LOOKING ON THE BRIGHT SIDE

The neural basis of emotion processing and regulation in groups at increased risk for psychosis

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1 General introduction

Many situations in daily life elicit negative emotions. For example, a flat tire may cause frustration and anger and deadlines may result in stress and anxiety. Our initial response to these situations is based on the way we perceive and process the emotional content of the situation. Whether or not we keep this initial emotional state is highly dependent on how we regulate our emotions (Gross, 1998). Cognitive, social and emotional functioning may all be severely impaired in people with schizophrenia, a severe psychiatric disorder which is further characterized by psychotic symptoms, such as hallucinations and delusions (Mueser and McGurk, 2004). Over the last two decades, research on emotion dysfunction in schizophrenia has increased exponentially and revealed impairments in emotion perception and expression, in the face of intact experience of emotions (Aleman and Kahn, 2005; Kohler et al., 2010; Kring and Elis, 2013). Furthermore, studies have indicated emotion dysregulation in schizophrenia (e.g. van der Meer et al., 2009). However, it still remains an open question whether the impairments in emotion processing and regulation are more disease-related or part and parcel of the vulnerability to the disorder. Therefore, the aim of this thesis was to examine whether difficulties with emotion processing and emotion regulation are already present in groups at increased risk for developing psychosis. More specifically, the neural mechanisms, both structural and functional, underlying these processes were examined through (functional) Magnetic Resonance Imaging (fMRI) in three groups with an increased risk for psychosis. These three groups consisted of subjects with high levels of alexithymia, subjects at increased genetic risk for schizophrenia and subjects at ultra-high risk (UHR) for developing psychosis.

|THE CLINICAL CHARACTERISTICS OF SCHIZOPHRENIA

Schizophrenia is a severe mental illness characterized by positive symptoms, such as delusions and hallucinations, negative symptoms (e.g. blunted affect, poverty of speech and apathy), disorganized behavior and cognitive impairment (Mueser and McGurk, 2004). The lifetime prevalence of schizophrenia is approximately 0.4% worldwide (McGrath et al., 2008). Of these patients only about 14% will recover (e.g. symptom-free) (Jaaskelainen et al., 2013), indicating that schizophrenia is a chronic disorder. It is often suggested that schizophrenia should be regarded a cognitive disorder characterized by dysfunction in, amongst others, memory and attention (Elvevag and Goldberg, 2000; Kahn and Keefe, 2013). However, the last two decades research is also increasingly focusing on emotional dysfunctions in schizophrenia (Aleman and Kahn, 2005).

|EMOTION PROCESSING AND EMOTION REGULATION

Emotion processing is not a unitary construct, it comprises several phases which can be described according to the process model of emotion elicitation (Smith and Kirby, 2000). First, when an emotional stimulus is perceived, appraisal detection takes place in which the system becomes alert. During appraisal detection, the amygdala gets activated and directs attention toward the stimulus by activating sensory areas, such as the occipital cortex (Adolphs, 2002a; Vuilleumier, 2005). Simultaneously, the perceived emotional stimulus might trigger associated knowledge, such as memories via activation of the hippocampal formation (Adolphs, 2002b), which may influence the appraisal detection. For example, when seeing a dog, the memory of being bitten by a dog last month may result in a stronger appraisal detection (i.e. stronger amygdalar activation).

After appraisal detection, appraisal integration takes place in which an emotional response is formed. This emotional response consists of physiological activation, such as an increased heart rate and action tendencies (Smith and Kirby, 2000). If this emotional response is strong enough, a person will become aware of these responses and a subjective affective state of emotional feeling will be formed (Smith and Kirby, 2000). Activation of the medial prefrontal cortex, orbitofrontal cortex and insula is thought to underlie the generation of these emotional feelings (Kober et al., 2008; Phillips et al., 2003). This generated emotional feeling can be very helpful. For example, when seeing a poisonous snake, it is useful to feel frightened as it warns you not to approach the snake. However, in other situations, reacting to our initial emotional response can do more harm than good (Gross, 2002). For instance, starting a fight when someone is badmouthing you might not always be the best solution. In these situations applying emotion regulation to change the emotions we are experiencing and the way we express them can be beneficial (Gross, 1998).

Two widely studied and often applied emotion regulation strategies are cognitive reappraisal and expressive suppression (Gross, 1998). Cognitive reappraisal can be described as the reappraisal of emotional stimuli in such a way that the emotional content decreases (Gross, 1998), either by reinterpretation or by distancing. For example, when seeing a woman cry in front of a church, one could think her daughter just got married and that the tears are actually tears of joy instead of thinking the woman attended a funeral (i.e. reinterpretation). Or, when viewing a picture of a crying child, one could view the image from a detached perspective by thinking it is a movie pamphlet and not a real situation (i.e. distancing). During reappraisal, prefrontal brain regions, including the dorsolateral, dorsomedial and ventrolateral prefrontal cortex (DLPFC, DMPFC, VLPFC), as well as the posterior parietal cortex and the middle temporal gyrus become activated (for meta-analyses see, Buhle et al., 2013; Diekhof et al., 2011). These regions are thought to be responsible for the cognitive regulation of emotion (Ochsner et al., 2012). More specifically, the DLPFC and the parietal cortex may direct attention to reappraisal-relevant features of the stimulus (Ochsner et al., 2012), while the DMPFC might be involved in analyzing the affective meaning of emotional stimuli (Ochsner and Gross, 2005). Furthermore, the VLPFC is thought to modulate and inhibit the emotional appraisals (Ochsner et al., 2002). Via connections from the prefrontal cortex to the limbic system, the increased prefrontal activation results in lower activation in the limbic system (e.g. amygdala, ventral striatum), resulting in a dampening of the appraisal detection and subsequently, lower negative affect (Diekhof et al., 2011; Ochsner et al., 2012; Smith and Kirby, 2000). Besides these brain activation patterns, successful application of cognitive reappraisal is also related to structural brain differences such as higher gray matter volume in the anterior cingulate cortex (ACC), orbitofrontal cortex and superior temporal gyrus (Giuliani et al., 2011b; Mak et al., 2009).

Expressive suppression can be described as the inhibition of emotion-expressive behavior (i.e. not showing how you feel by keeping a poker face) (Gross, 1998). The neuroimaging data on expressive suppression is rather scarce compared to the reappraisal literature. A possible reason for this might be that correct application of expressive suppression may be hard to verify without electromyographic data or recordings of the emotional expressions. Studies that did investigate suppression showed increased activation in, amongst others, the DLPFC, precentral gyrus, supplementary motor area, premotor cortex, supramarginal gyrus, and temporal regions during suppression (Goldin et al., 2008; Hayes et al., 2010; Vanderhasselt et al., 2012; Vrticka et al., 2011; Vrticka et al., 2013). Furthermore, subjects who report more use of expressive suppression have higher gray matter volume in the insula and DMPFC (Giuliani et al., 2011a; Kuhn et al., 2011). These regions are involved in inhibitory motor control and, therefore, might be responsible for the reduction of emotional expression

during expressive suppression (Goldin et al., 2008; Vanderhasselt et al., 2012; Vrticka et al., 2011).

Cognitive reappraisal and expressive suppression are associated with different outcomes. Cognitive reappraisal results in higher positive affect and more expression of positive emotions, while negative affect and the expression thereof are reduced (Gross, 2002). In contrast, expressive suppression results in less emotional expression, lower positive affect and either no changes or increases in negative affect (Gross, 2002). Furthermore, there is a positive association between the use of reappraisal and reported life satisfaction, well-being and physical health, while suppression is negatively associated with these factors (Appleton et al., 2014; Gross, 2002). Therefore, cognitive reappraisal can be seen as a more effective method of emotion regulation in comparison to expressive suppression.

|SCHIZOPHRENIA AND EMOTION REGULATION

Patients with schizophrenia may be impaired in the processing of emotions. For example, patients with schizophrenia attribute salience to non-salient stimuli (Cohen and Minor, 2010). Higher activation in the amygdala in response to neutral stimuli is suggested to underlie this aberrant appraisal detection (Hall et al., 2008). Furthermore, patients show restricted emotional expression, not only in their facial expression (affective flattening, Kring & Neale, 1996), but also in tone of voice or emotion prosody (Hoekert et al., 2007), while the experience of emotions appears to be intact or may be even heightened (Kring and Elis, 2013). During emotion labeling and recognition tasks, patients with schizophrenia often underperform compared to controls (Kohler et al., 2010). Meta-analyses have shown that this lower performance might be caused by lower activation in emotion processing regions, such as the ACC, DMPFC, caudate, parahippocampal gyrus, and fusiform gyrus during emotion recognition tasks (Li et al., 2010; Taylor et al., 2012).

Besides the emotion recognition difficulties, patients may be impaired in their regulation of emotion. For example, patients with schizophrenia tend to use less cognitive reappraisal (Kimhy et al., 2012; Livingstone et al., 2009; van der Meer et al., 2009), while applying more expressive suppression (Kimhy et al., 2012; van der Meer et al., 2009), although not all studies have replicated this (Henry et al., 2008; Perry et al., 2011). Furthermore, recent neuroimaging studies have indicated that patients activate the DLPFC and VLPFC to a lesser extent compared to controls during reappraisal (Morris et al., 2012; van der Meer et al., 2014), while being less capable of reducing negative affect through reappraisal (Morris et al., 2012).

Difficulties in cognitive and emotion processing might not solely be related to functional differences in brain activation. Meta-analyses have shown that in patients with schizophrenia, gray matter is lower in various regions such as the thalamus, insula, amygdala, superior temporal gyrus, fusiform gyrus, medial frontal gyrus and inferior frontal gyrus (Ellison-Wright et al., 2008; Fornito et al., 2009; Glahn et al., 2008). All these regions play an important role in the processing and regulation of emotions (Adolphs, 2002a; Buhle et al., 2013; Phillips et al., 2003). Therefore, gray matter reductions in these regions may also underlie the emotion processing and regulation deficits in schizophrenia.

Taken together, the literature indicates emotion processing and regulation difficulties in patients with schizophrenia. Abnormalities in both brain structure and function are probably underlying these difficulties. However, the question remains whether these difficulties and associated brain abnormalities are related to schizophrenia itself or if they are part of the

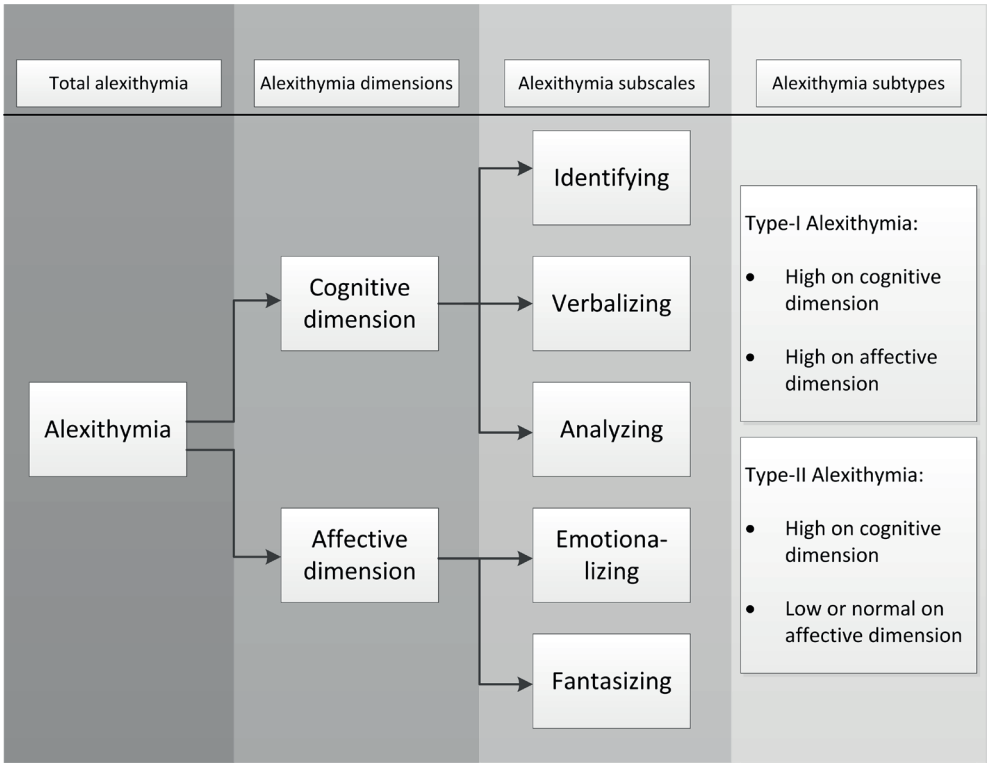
vulnerability to psychosis. This question is of great relevance as emotion dysregulation is associated with higher levels of negative affect, distress and social exclusion (Butler et al., 2003; Gross, 2002). These three factors have been reported to precede psychotic symptoms, such as delusions (Freeman and Garety, 2003; Hanssen et al., 2005). Furthermore, in subjects at high risk for developing psychosis, due to subclinical hallucinations, comorbid depressive feelings can even enhance the chance of developing a psychotic episode (Krabbendam et al., 2005). It has been suggested to provide reappraisal training in patients and subjects at high risk for psychosis to reduce negative affect and possibly prevent psychotic episodes (Hanssen et al., 2005; Krabbendam et al., 2005). However, to our knowledge, it has not yet been examined whether subjects at high risk for developing psychosis indeed experience emotion regulation difficulties.

|GROUPS AT INCREASED RISK FOR DEVELOPING SCHIZOPHRENIA

The etiology of schizophrenia is still unclear, due to the complex nature of the disorder. However, research has shown that the disorder is in part heritable (Cardno et al., 1999) and several risk factors, such as birth complications, urban environment, immigration and adverse life events have been identified (McDonald and Murray, 2000). Studying individuals at increased risk for developing schizophrenia can provide useful information in discovering the causes of schizophrenia and may improve the early detection of the disorder. The latter is of great importance because clinical symptoms, especially negative symptoms, worsen during the course of schizophrenia and the longer the illness is untreated, the poorer the outcome is on clinical symptoms and social functioning (Mueser and McGurk, 2004). Another advantage of studying individuals at increased risk for psychosis is that the results are not influenced by possible toxic effects of psychotic episodes and/or medication use, as is often the case when studying patients with schizophrenia. Furthermore, studying groups with an increased risk for psychosis can help identify possible endophenotypes for psychotic disorders. Endophenotypes are measureable components that can make the connection between predisposing genes and the disorder itself and may provide clues to the genetic underpinnings of certain disorders (Gottesman and Gould, 2003). Two of the criteria for identifying endophenotypes can be examined by studying subjects with an increased vulnerability for psychosis: 1) endophenotypes should be present in non-affected family members of patients and 2) endophenotypes should be state-independent (i.e. already present before the onset of the disorder) (Gottesman and Gould, 2003). Therefore, in this thesis, we focus on three groups that are at (putative) increased risk for developing psychotic disorders (e.g. schizophrenia), namely individuals with high scores on alexithymia, siblings of patients with schizophrenia and individuals at UHR for developing psychosis.

Alexithymia

Alexithymia ("no words for feelings") is a personality construct that denotes difficulties in identifying, verbalizing and analyzing one's feelings and in distinguishing them from bodily sensations of arousal. Furthermore, individuals with high scores on alexithymia show a lack of imagination (Sifneos, 1973; Vorst and Bermond, 2001). Alexithymia is mostly assessed through self-report questionnaires of which the Bermond-Vorst alexithymia questionnaire [BVAQ; (Vorst and Bermond, 2001)] and the Toronto alexithymia scale [TAS-20; (Bagby et al., 1994)] are the most frequently used. Alexithymia can be divided in a cognitive and an affective dimension (Bermond et al., 2007). The cognitive dimension consists of the



|Figure 1.1 Schematic representation of the alexithymia dimensions, subscales and alexithymia types

subscales, difficulties in identifying, verbalizing and analyzing feelings, while the affective dimension consists of the subscales emotionalizing (i.e. the extent to which individuals get emotionally aroused) and fantasizing (i.e. the extent to which individuals are inclined to fantasize and daydream). Based on these alexithymia dimensions, two types of alexithymia can be distinguished. Type-I alexithymia is characterized by high scores on both the cognitive and affective alexithymia dimension, indicating low levels of emotional arousal and impaired emotional cognitions. Type-II alexithymia, on the other hand, is characterized by high scores on the cognitive alexithymia dimension, but low to normal scores on the affective dimension. This indicates normal or even high levels of emotional arousal, while the cognitions accompanying these emotions are impaired. The different alexithymia dimensions and types are schematically represented in Figure 1.1.

With a prevalence rate of ten percent in the general population (Salminen et al., 1999), alexithymia is a putative risk factor for a range of psychiatric disorders such as anxiety disorders, depression, somatoform disorders and schizophrenia (Taylor et al., 1997). Although no longitudinal studies have been performed to examine this proposed risk, there are some indications that individuals with high alexithymia scores might be at increased risk for developing schizophrenia. Previous literature has shown that patients with schizophrenia have indeed higher levels of alexithymia (Kubota et al., 2011; Kubota et al., 2012; Yu et al., 2011). More specifically, patients score higher on the cognitive alexithymia dimension while scores on the affective dimension are equal (van der Meer et al., 2009) or lower (van ‘t Wout et al., 2007) compared to healthy controls. Thus, whereas they have more difficulties

identifying and verbalizing their feelings, they may at the same time have normal or heightened levels of emotional arousal. Furthermore, it has been suggested that alexithymia might be a vulnerability marker for schizophrenia as higher scores on alexithymia are already present in groups at increased risk for schizophrenia (van 't Wout et al., 2007; van Rijn et al., 2011).

Emotion processing difficulties are at the core of alexithymia. For example, individuals with alexithymia show difficulties with the identification of facial expressions (Grynberg et al., 2012), difficulties with emotion recognition in general (Lane et al., 2000), problems with emotional memory (Luminet et al., 2006) and impaired higher order mentalizing (Swart et al., 2009). These emotion processing difficulties are also reflected in abnormal brain activation patterns. Research has reported that during emotion processing, individuals with high scores on alexithymia show aberrant activation patterns in emotion processing regions such as, the ACC, insula, amygdala and prefrontal cortex compared to low scoring individuals (Bermond et al., 2006; Lane et al., 1997; Taylor and Bagby, 2004). In order to identify which brain regions are underlying the emotion processing difficulties in alexithymia, we performed a meta-analysis integrating the neuroimaging literature on emotion processing in alexithymia (**chapter 2**). Besides functional (i.e. brain activation) differences, structural (i.e. brain volume) differences in the brain might also be underlying the emotion processing difficulties in alexithymia. Therefore, we examined the volume of gray and white matter in subjects differing in alexithymia scores (**chapter 3**). Specifically, we examined whether the cognitive and affective alexithymia dimensions would be differentially related to brain volume.

Alexithymia is considered to represent deficient emotion regulation (Aleman and Kahn, 2005; Taylor et al., 1997; Taylor and Bagby, 2004). Several studies have supported this claim by showing that individuals with alexithymia apply less reappraisal and more suppression compared to non-alexithymic controls (Kessler et al., 2010; Stasiewicz et al., 2012; Swart et al., 2009; Wingenfeld et al., 2011). This is the same pattern as seen in patients with schizophrenia (e.g. van der Meer et al., 2009). However, no neuroimaging studies had yet examined the neural correlates of emotion regulation in subjects with alexithymia. Therefore, we applied an emotion regulation task to examine brain activation related to alexithymia during cognitive reappraisal. The results are presented in **chapter 4**.

As described above, previous studies have shown that higher scores on alexithymia are present in both patients (van der Meer et al., 2009) as well as individuals at high risk for psychosis (van 't Wout et al., 2007; van Rijn et al., 2011). However, these studies did not compare alexithymia scores between subjects at a high genetic risk for psychosis (e.g. siblings of patients with schizophrenia) and subjects at UHR for psychosis (both groups are described in more detail below). Therefore, we examined whether alexithymia is related to the degree of risk for psychosis by comparing alexithymia scores between these high risk groups (**chapter 5**).

Siblings of patients with schizophrenia

Siblings of patients with schizophrenia are at a tenfold increased genetic risk for developing schizophrenia (Gottesman, 1991). This increased risk is reflected in impaired functioning as siblings of patients with schizophrenia show the same cognitive and emotion processing difficulties as patients, albeit to a lesser extent (Gur et al., 2007; Lavoie et al., 2013; Sitskoorn et al., 2004). For example, on group level, siblings seem to be less capable in identifying facial expressions (for meta-analysis see Lavoie et al., 2013) and higher levels of alexithymia have been reported in this group (van 't Wout et al., 2007). A number of

neuroimaging studies have reported that during emotion processing, siblings have lower activation in emotion processing areas, such as the amygdala, precentral gyrus and medial and superior frontal regions compared to controls (Habel et al., 2004; Li et al., 2012; Lo Bianco et al., 2013; Venkatasubramanian et al., 2010). However, increased activation in, amongst others, the amygdala, hippocampus, medial prefrontal cortex and middle cingulate gyrus during emotion processing has also been reported in siblings (van Buuren et al., 2011).

Besides brain activation differences, previous studies have reported structural abnormalities in emotion processing brain areas in siblings of patients with schizophrenia (Boos et al., 2007; Fusar-Poli et al., 2014b; Palaniyappan et al., 2012). However, the results reported in the literature are largely inconsistent and several large studies reported no differences at all (Boos et al., 2012; Honea et al., 2008; Job et al., 2003). To examine whether these divergent findings could be explained by differences in age, genetic loading and schizotypy, we performed a structural MRI-study to examine gray matter abnormalities in siblings of patient with schizophrenia. The results of these analyses are presented in **chapter 6**.

The structural and functional differences in siblings are not solely restricted to early emotion processing regions. Areas involved in emotion regulation, such as the superior and medial frontal gyrus, have also been reported to show lower gray matter volume (Palaniyappan et al., 2012) and abnormal activation patterns (van Buuren et al., 2011; Venkatasubramanian et al., 2010) in relatives of patients with schizophrenia compared to controls. This indicates that these abnormalities might not only be related to the early phases of emotion processing, but might also extent to the later phase of emotion regulation. To this extent, we examined the neural correlates of emotion regulation in siblings of patients with schizophrenia (**chapter 7**).

Ultra-high risk group

The onset of schizophrenia is often preceded by a prodromal phase, also called the At-Risk Mental State (ARMS). During this phase, ARMS individuals experience subclinical psychotic symptoms and a decline in social functioning (Yung and McGorry, 1996). Individuals who meet the criteria for ARMS (see Table 1.1) are at ultra-high risk (UHR) for developing psychosis, with transition rates of 29% after two years (Fusar-Poli et al., 2012). Several terms have been applied to describe this group, such as ARMS, UHR and clinical high risk (CHR). In this thesis the term UHR will be used. Previously, the focus in UHR literature has been mainly on difficulties in cognitive processing. For example, research reported UHR subjects to have more cognitive impairments than controls (for overview see Woods et al., 2010) and show lower activation in the ACC, VLPFC, DMPFC and parietal cortex during cognitive tasks (i.e. N-back and oddball-task) (Fusar-Poli et al., 2010; Fusar-Poli et al., 2011b; Morey et al., 2005). More recently, literature has also indicated that subjects at UHR for psychosis perform worse at facial and vocal emotion recognition tasks (Addington et al., 2012b; Amminger et al., 2012a; Amminger et al., 2012b; Comparelli et al., 2013) and show more amygdala activation and less VLPFC activation during emotion processing (Gee et al., 2012).

Both the cognitive and affective neuroimaging literature points to lower brain activation in the UHR group. Although most of these findings are related to non-emotional processing, the ACC, VLPC and DMPFC are also involved in emotion regulation (Buhle et al., 2013) and reappraisal can be seen as a cognitive emotional task (Ochsner and Gross, 2005). Furthermore, structural studies have shown lower gray matter volume in these regions in subjects at UHR for psychosis (Fusar-Poli et al., 2011a). Together with the emotion regulation

|Table 1.1 At risk mentral state criteria

Criteria
1. An impairment in social functioning SOFAS score of ≥ 50 or a 30% drop in SOFAS scores (Goldman et al., 1992)
2. Part of at least one of these three subgroups
a. Genetic vulnerability At least one first-degree relative with a history of psychosis or with a schizotypal personality disorder
b. Attenuated symptom group Intensity and frequency scores within the specified range (see CAARMS manual) on one or more attenuated positive symptoms
c. Brief limited intermittent psychotic syndrome (BLIPS) Intensity and frequency scores above the cut-off of psychosis (see CAARMS manual) on one or more of the positive symptoms Symptoms are present for less than one week and remit spontaneously

Rietdijk et al. (2010); *Abbreviations*: CAARMS: Comprehensive Assessment of At-risk Mental States; SOFAS: Social and occupational functioning assessment scale

difficulties reported in patients (e.g. van der Meer et al., 2009), this suggests that subjects at UHR for psychosis might also be impaired at emotion regulation. To examine this, we performed an fMRI-study including UHR subjects to examine the use and neural correlates of reappraisal in this group (**chapter 8**).

In sum, the work presented in this thesis aims to contribute to the knowledge of the neural basis of emotion processing and regulation in subjects at 'high risk' for psychosis. Identifying emotion processing and regulation difficulties in high risk groups is of great importance as these difficulties may increase the vulnerability to psychosis. Understanding the neural basis of these problems will give insight in the underlying mechanisms of emotion dysregulation in subjects at increased risk for psychosis and may lead to more targeted interventions in the future. In **part I** of this thesis we will focus on subjects with a putative high risk for psychosis due to high alexithymia scores. In **part II** we focus on siblings of patients with schizophrenia from the Genetic Risk and Outcome of Psychosis study (see Box 1.1) with an increased genetic risk for psychosis. In **part III** we present the results regarding subjects at UHR for developing psychosis. In the final chapter (**chapter 9**), the findings of the presented studies will be integrated and discussed. Furthermore, suggestions for future research and clinical implications will be given.

Box 1.1: The Genetic Risk and Outcome of Psychosis study

The Genetic Risk and Outcome of Psychosis (GROUP) study is a longitudinal multi-center study. The aim of the GROUP-study is to examine the genetic and non-genetic risk and resilience factors for non-affective psychotic disorders. The GROUP-study is a collaboration of the Academic Medical Center of Amsterdam, the University Medical Center Groningen, the University Medical Center Utrecht and the Maastricht University Medical Center. At baseline, 1120 patients, 1057 siblings, 919 parents and 590 healthy controls were included. For a complete overview of the objectives and methods of the GROUP-study, see Korver et al. (2012).

Add-on study

In collaboration with the Free University of Amsterdam and the Academic Medical Center of Amsterdam, an add-on study of the GROUP-study was performed. In this add-on study, 98 healthy siblings and 85 healthy controls were included. The aim of this add-on study was to examine the neural correlates of cognitive and emotion processing in subjects at genetic risk for developing schizophrenia. Furthermore, the neural correlates in relation to several genetic polymorphisms were investigated.

PART I
ALEXITHYMIA

2 Neural correlates of alexithymia: A meta-analysis of emotion processing studies

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|ABSTRACT

BACKGROUND: Alexithymia is a personality trait characterized by difficulties in the experience and cognitive processing of emotions. It is considered a risk factor for a range of psychiatric and neurological disorders. Functional neuroimaging studies investigating the neural correlates of alexithymia have reported inconsistent results.

METHODS: To integrate previous findings, we conducted a parametric coordinate-based meta-analysis including fifteen neuroimaging studies on emotion processing in alexithymia.

RESULTS: During the processing of negative emotional stimuli, alexithymia was associated with a diminished response of the amygdala, suggesting decreased attention to such stimuli. Negative stimuli additionally elicited decreased activation in supplementary motor and premotor brain areas and in the dorsomedial prefrontal cortex, possibly underlying poor empathic abilities and difficulties in emotion regulation associated with alexithymia. Positive stimuli elicited decreased activation in the right insula and precuneus, suggesting reduced emotional awareness in alexithymia regarding positive affect. Independent of valence, higher (presumably compensatory) activation was found in the dorsal anterior cingulate possibly indicating increased cognitive demand.

CONCLUSION: These results suggest valence-specific as well as valence-independent effects of alexithymia on the neural processing of emotions.

|INTRODUCTION

Alexithymia ("no words for feelings") is a personality trait characterized by difficulties in identifying, analyzing and verbalizing feelings, restricted imaginal capacities and limited emotional experience (Sifneos, 1973; Vorst and Bermond, 2001). Its prevalence rate lies around 10% in the general population (Salminen et al., 1999). In the past, there has been some debate on whether alexithymia should be conceptualized as a distinct clinical type or as a dimensional personality construct. However, recent studies provided strong support in favor of alexithymia as a dimensional personality construct (Mattila et al., 2010; Parker et al., 2008). Alexithymia is considered to be a risk factor for various psychiatric and psychosomatic disorders, including substance abuse, depression and schizophrenia (Taylor et al., 1997; van 't Wout et al., 2007). Furthermore, individuals with alexithymia report lower life satisfaction (Mattila et al., 2007) and are more likely to commit suicide (Hintikka et al., 2004). Therefore, it is of great clinical importance to gain more insight in the neural basis underlying this personality trait.

Difficulties in emotion processing are at the core of alexithymia. Both the ability to experience and cognitively process emotions is reduced. For example, individuals with alexithymia show impaired performance in remembering emotional words (Luminet et al., 2006), problems during the identification of facial expressions (Grynberg et al., 2012; Parker et al., 1993) and impaired higher order mentalizing (Swart et al., 2009). Hence, theories on neural correlates of alexithymia mainly focus on brain areas involved in emotion processing. One theory states that alexithymia might be associated with a right hemisphere deficit or a left hemisphere preference (Bermond et al., 2005; Buchanan et al., 1980) because the right hemisphere plays an important role in the perception and regulation of emotional behavior (Adolphs et al., 2000).

Lane and colleagues (1997) hypothesized a central role for the anterior cingulate cortex (ACC) in alexithymia. According to their 'blindfeel' hypothesis, the conscious experience of emotion is compromised in individuals with alexithymia, assumed to result from a dysfunction in the ACC (Lane et al., 1997). In addition to the ACC, the insula is another relevant brain region in generating emotional experience. This structure receives information from internal bodily states and integrates these into a subjective feeling state (Craig, 2009). Furthermore, subcortical areas, such as the amygdala and striatum, are proposed to underlie emotion processing difficulties in alexithymia because of their role in the detection of emotional significance and the generation of emotional feelings (Bermond et al., 2006; Kano and Fukudo, 2013; Larsen et al., 2003; Moriguchi and Komaki, 2013; Taylor and Bagby, 2004; Wingbermühle et al., 2012). Thus, alexithymia-related difficulties in perceiving and experiencing emotions may be associated with dysfunction of the ACC, insula, amygdala and striatum. According to two recent reviews, this decrease in limbic and paralimbic activation is associated with a decrease in prefrontal activation when individuals with high alexithymia scores are presented with external emotional stimuli (Kano and Fukudo, 2013; Moriguchi and Komaki, 2013). Especially, altered activation in the orbitofrontal cortex and medial prefrontal cortex is proposed to underlie alexithymia (Bermond et al., 2006; Larsen et al., 2003; Wingbermühle et al., 2012) because of their involvement in the cognitive control of emotions, including emotion regulation (Ochsner and Gross, 2005) and emotional decision making (Rogers et al., 2004). In fact, lesions in these regions have been shown to result in restricted capacities for the cognitive processing of emotions (Glascher et al., 2012). Difficulties in the restricted imaginal capacities in alexithymia, on the other hand, are thought to be related to reduced activation in the posterior cingulate cortex (Aleman, 2005; Bermond et al., 2006; Kano and Fukudo, 2013; Larsen et al., 2003; Moriguchi and Komaki, 2013;

Wingbermühle et al., 2012) because of its role in emotional memory (Maddock, 1999) and the imagination of future events (D'Argembeau et al., 2008).

To date, neuroimaging studies have tried to identify these proposed brain regions as neural correlates of alexithymia. In 2010, Pouga and colleagues compared alexithymia-related brain activation in the medial frontal gyrus, cingulate gyrus and amygdala across studies. They identified lower activation in the medial frontal gyrus and the amygdala in alexithymia, while results on the cingulate gyrus were mixed (i.e. both higher and lower activation was associated with alexithymia). Furthermore, results regarding other brain areas in alexithymia are also inconsistent across studies. Most existing reviews on the neural correlates of alexithymia highlight the variability in findings across studies but do not integrate these findings (Aleman, 2005; Bermond et al., 2006; Larsen et al., 2003; Taylor and Bagby, 2004; Wingbermühle et al., 2012). Furthermore, only a select group of brain regions are mentioned in these reviews, leaving other possible neural correlates unremarked.

The aim of the present study was to integrate findings from the literature and identify brain regions underlying emotion processing difficulties in alexithymia across studies. Therefore, studies examining the neural correlates of alexithymia during the processing of either positive or negative emotional stimuli were included in this meta-analysis. Positive and negative emotional processing presumably differ in their neural correlates (Wager et al., 2003). Furthermore, previous studies have indicated that there appears to be a valence-specific effect on the neural correlates of emotion processing in alexithymia (Berthoz et al., 2002; Kano et al., 2003; Pollatos and Gramann, 2011; Reker et al., 2010). For example, studies investigating alexithymia reported decreased activity in the amygdala for negative, but not for positive stimuli (Kugel et al., 2008; Reker et al., 2010). Moreover, Berthoz et al. (2002) reported decreased activation in the ACC for negative stimuli, while activation in this area was increased for positive stimuli in alexithymia. Besides these indications of possible differences between the neural correlates of positive and negative emotion processing in alexithymia, a recent meta-analysis indicated that emotional valence modulates neural abnormalities in depression (Groenewold et al., 2012). Given the fact that alexithymia is significantly related to depression (Honkalampi et al., 2000), one could hypothesize that valence might also moderate alexithymia-related brain activation. Therefore, brain activation associated with alexithymia was examined for the processing of negative and positive stimuli separately. A novel parametric coordinate-based meta-analysis (PCM) approach (Costafreda, 2012) was employed as this method allowed for the inclusion of both whole brain and region of interest (ROI) studies as well as for the inclusion of different thresholds across studies.

|METHODS

Systematic Literature Search

Studies investigating the neural basis of alexithymia, published before April 2013, were identified through a systematic literature search in the PubMed and Web of Knowledge databases. The search keywords were "alexithymia" OR "alexithymic" combined with MRI OR magnetic resonance imaging OR fMRI OR functional magnetic resonance imaging OR PET OR positron emission tomography OR neuroimaging OR brain imaging OR imaging OR BOLD OR blood oxygen level dependent. In addition, the references of the included manuscripts and previously published reviews (Aleman, 2005; Bermond et al., 2006; Lane et al., 1997; Larsen et al., 2003; Taylor, 2000; Taylor and Bagby, 2004; Wingbermühle et al., 2012) were manually scanned to obtain eligible studies not identified through the initial search.

Inclusion criteria were (a) the use of fMRI or PET, (b) the assessment of brain activation during emotion processing related to alexithymia, (c) the use of image subtraction to identify brain areas associated with either negative or positive emotional stimuli versus neutral stimuli or versus a neutral baseline and, (d) the assessment of alexithymia using the Toronto Alexithymia Scale (TAS-20) or the cognitive dimension of the Bermond-Vorst Alexithymia Questionnaire (BVAQ). Both the TAS-20 and the BVAQ are self-report measures frequently applied to assess alexithymia in current neuroimaging studies. The TAS-20 assesses the cognitive dimension of alexithymia while the BVAQ assesses both the cognitive and affective dimension of alexithymia. Because the cognitive dimension of the BVAQ and the TAS-20 total score are highly correlated (Vorst and Bermond, 2001), studies applying either the TAS-20 or the cognitive dimension of the BVAQ were included in this meta-analysis. The affective dimension of the BVAQ could not be included in the current meta-analysis, since only one study examined this dimension (Pouga et al., 2010). Neuroimaging studies applying the Level of Emotional Awareness Scale (LEAS) were excluded from the analyses as well because this scale was not developed to examine alexithymia per se (Lane et al., 1998). Furthermore, the LEAS is only weakly or non-significantly correlated with the TAS-20 (Lumley et al., 2005) and the BVAQ (Fantini-Hauwel et al., 2012). Studies were further excluded if (a) the articles were not written in English, (b) no original data was presented (e.g. review, letter or comment), (c) only an abstract was published or (d) the study sample consisted of patients only or patients and healthy controls were combined in one sample. Two researchers (JV and MS) independently performed the data search and selection. Discrepancies were resolved by consensus. When necessary, corresponding authors of the selected papers were contacted to clarify the methods and/or results.

Data extraction

The following data were extracted from each study: (1) sample size, (2) contrasts examined (negative versus neutral/baseline, positive versus neutral/baseline, positive or negative correlation with alexithymia), (3) field of view (ROI or whole brain), (4) the Automated Anatomical Labeling (AAL) (Tzourio Mazoyer et al., 2002). In case a ROI analysis was performed based on a mask created according to the AAL system and no information was given on the coordinates, the AAL region was fed into the analyses. When the ROI region was not defined according to the AAL system, the region was relabeled according to the AAL system by the authors in consultation with a trained anatomist. Information on the included ROIs and the AAL labels can be found in supplementary Table S2.1, (5) normalization template (MNI or Talairach), (6) location information (the sets of x, y, z brain coordinates for significant findings or an empty x, y, z set for non-significant findings), (7) study-specific statistical threshold, (8) information on statistical significance (p, Z, T, F) and the corresponding value and, (9) the applied smoothing kernel. The data were extracted by the first author and checked independently by another researcher (MS).

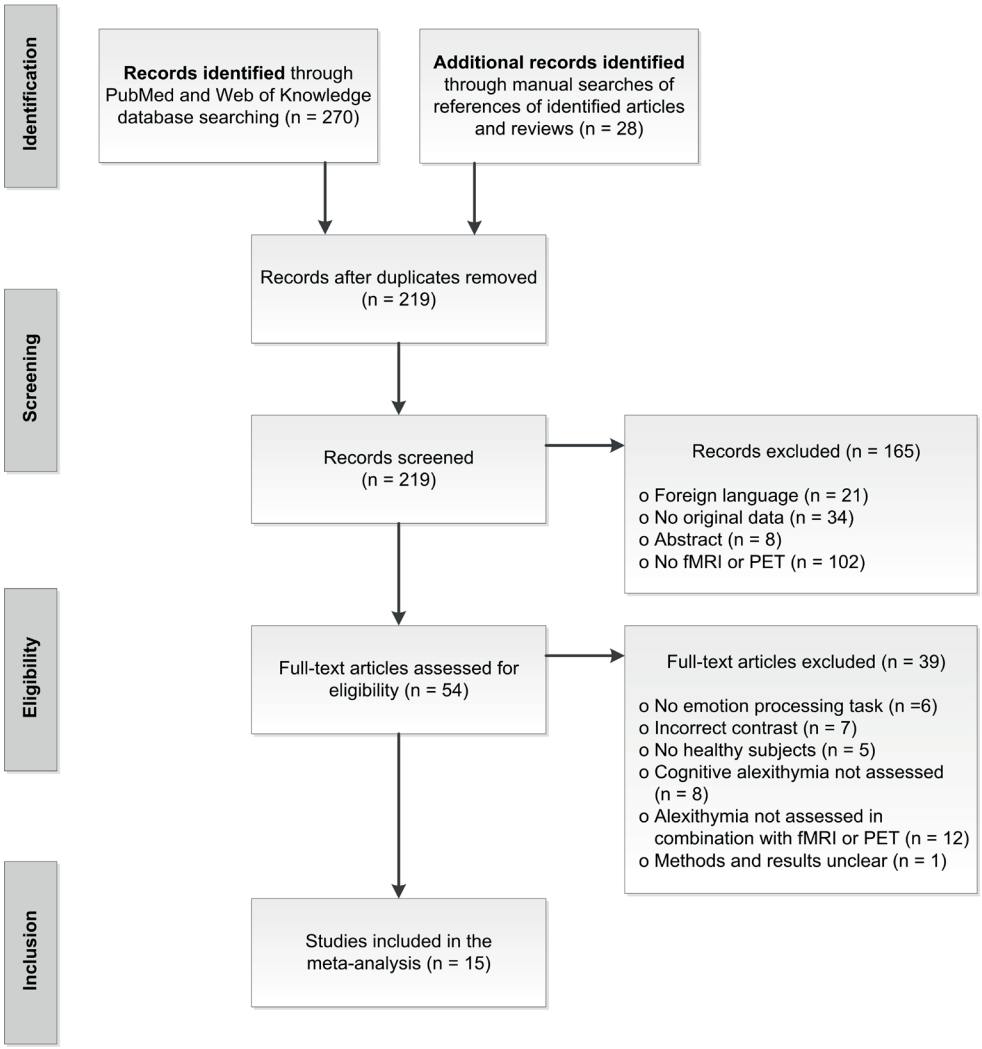
Data-analysis

To summarize the results reported across studies, software was used that implements a new parametric coordinate-based meta-analysis (PCM) technique allowing the pooling of both ROI-based and coordinate-based findings (described in detail in Costafreda, 2012). In neuroimaging meta-analyses, differences in statistical thresholds across studies that are being summarized are frequently ignored. However, statistical thresholds can vary

substantially, from strict family-wise correction methods to uncorrected p-values. The key advantage of the PCM approach to neuroimaging meta-analyses is that it produces valid effect-size summaries of studies with different statistical thresholds. Briefly, an unbiased voxel-wise statistical summary map is produced based on the spatial location and effect size of the maxima of significant as well as non-significant findings.

First, when necessary, coordinates were transformed from Talairach on the MNI coordinate system by using a non-linear transformation (Brett et al., 2001). Second, all of the effect sizes and statistical threshold values (i.e. p, T, r or F) were converted into Z-values as a common measure of effect size. Next, Z-value summary maps were created for each study by convolving the significant findings of a study with a uniform kernel size of 15 mm. In other words, the Z-value associated with a significant finding at a specific coordinate (x, y, z) was distributed across all voxels within the 15 mm radius of the coordinate. This kernel width was chosen based on empirical evaluations which found that uniform kernels of 15-20 mm offer optimal sensitivity in neuroimaging meta-analyses (Radua et al., 2012; Wager et al., 2004). Non-significant findings were represented by intervals containing the values for the unknown measurements that were below the reported statistical thresholds. Such an interval estimate is analog to how a confidence interval in standard statistical estimation contains a range of probable values for an unknown population parameter. That is, the measurements in voxels located outside the radial distance in the whole-brain or ROI contrast were interval estimates with interval boundaries, determined by the statistical threshold reported by the study (e.g. a non-significant finding with an uncorrected threshold $p<.001$ is approximately equivalent to a Z interval of [-Inf, 3.09]).

Summary maps were created for the contrast 'positive>neutral' and 'negative>neutral' separately. Positive Z-values reflected a positive correlation with alexithymia or greater brain activation in high alexithymic individuals compared to low alexithymic individuals, while negative Z-values reflected negative correlations with alexithymia or greater activity in low alexithymic individuals compared to high alexithymic individuals. Fourth, an overall Z-value summary map was created by pooling the study level summary maps. The software created a pooled summary map by obtaining maximum likelihood estimates of the population mean and standard deviation of the Z-values across studies for each voxel through the optimization of the likelihood function of the normal distribution. The contribution of each study was weighted to the summary map by its sample size. Fifth, voxels in the summary map that had a Z-mean value significantly different from zero (i.e. the voxels showing evidence of differential brain activation) were determined by performing a two tailed t-test on the estimated Z-mean value for each voxel. The null hypothesis was: $|\mu|=0$ and the alternative hypothesis (H_1) was $|\mu|\neq 0$. Thus, voxels with a p-value below a chosen statistical threshold were deemed to show significant evidence to the alternative hypothesis. To correct for multiple comparisons, we used a $p<.05$ false discovery rate (FDR). Finally, the clusters of voxels which were larger than the chosen extent threshold of 100 mm^3 were determined. The thresholded (i.e. the Z-values (voxels) that survived the extent and statistical thresholds) T and r (correlation coefficient) effect size summary maps calculated from the Z-values were given as output. Two separate summary maps were given for the contrasts 'positive>neutral' and 'negative>neutral'. Clusters of voxels with a value>0 correlated positively with alexithymia while clusters of voxels with a value<0 correlated negatively with alexithymia.



|Figure 2.1 PRISMA flow diagram of study selection procedure

|RESULTS

The initial search returned 219 original titles. Of these, 165 were excluded after reviewing the abstracts and titles. Reasons for the exclusion of studies are presented in Figure 2.1. The full texts of the remaining 54 titles were examined in more detail, which resulted in the inclusion of 15 studies in this meta-analysis. For details on the inclusion procedure, see the flow chart in Figure 2.1. Details on the included studies are presented in Table 2.1 and 2.2. Specific reasons for exclusion of studies examining alexithymia-related brain activation can be found in supplementary Table S2.2.

Table 2.1 Details of studies included in the meta-analysis

Study	N	N male/N female	Mean age (SD)	Mean alexithymia scores (SD)	Questionnaire	Covariaties	Scanner method	Smoothing kernel (mm)
Reker et al. (2010)	33	0/33	24.8 (7.7)	37.9 (7.7)	TAS-20	BDI, STAI	fMRI 3T	6 x 6 x 6
Lee et al. (2011)	38	0/38	23.2 (3.5)	43.9 (10.0)	TAS-20	None	fMRI 1.5T	7 x 7 x 7
Eichmann et al. (2008)	22	14/8	27.9 (7.9)	43.9 (10.9)	TAS-20	None	fMRI 3T	6 x 6 x 6
Kugel et al. (2008)	21	13/8	26.5 (3.9)	43.2 (13.7)	TAS-20	BDI, STAI	fMRI 3T	6 x 6 x 6
Kano et al. (2003)	24 (12 HA; 12 LA)	24/0	HA: 23.2 (2.4) LA: 22.8 (1.7)	HA: 64.2 (3.6) LA: 40.5 (5.6)	TAS-20	None	PET	12 x 12 x 12
Mériau et al. (2006)	23	0/23	27.1 (4.7)	40.3 (6.7)	TAS	STAI, PANAS PA	fMRI 1.5T	8 x 8 x 8
Heinzel et al. (2010)	60 (30 HA; 30 LA)	60/0	HA: 26.6 (4.2) LA: 27.1 (4.8)	HA: 59.1 (5.4) LA: 33.3 (5.6)	TAS-20	BDI	fMRI 1.5T	8 x 8 x 8
Berthoz et al. (2002)	16 (8 HA; 8 LA)	16/0	21.5 (NS)	HA: 57.4 (8.9) LA: 33.6 (7.3)	TAS-20	None	fMRI 3T	7 x 7 x 7
Bird et al. (2010)	18	18/0	35.0 (12.8)	50.3 (14.5)	TAS-20	None	fMRI 1.5T	10 x 10 x 10
Moriguchi et al. (2007)	30	5/25	HA: 20.2 (1.0) LA: 20.8 (0.9)	HA: 66.1 (4.5) LA: 34.1 (3.7)	TAS-20	None	fMRI 1.5T	6 x 6 x 6
Noll-Hussong et al. (2013)	19	7/12	48.8 (12.3)	44.4 (8.6)	TAS-20	None	fMRI 3T	8 x 8 x 8
Kano et al. (2007)	45	34/11	22 (2)	47.8 (11)	TAS-20	None	PET	12 x 12 x 12
Strigo et al. (2013)	12	0/12	24.8 (6.1)	37.4 (7.2)	TAS-20	None	fMRI 3T	4 x 4 x 4
Pouga et al. (2005)	34 (13 HA; 12 LA; 9 I)	34/0	21.3 (2.4)	48.1 (10.9) TAS-20: 47.2 (12.5)	BVAQ-B	BDI, STAI	fMRI 3T	6 x 6 x 6
Mantani et al. (2005)	20 (10 HA; 10 LA)	14/6	HA: 25.9 (3.3) LA: 23.7 (3.0)	HA: 61.9 (4.0) LA: 37.9 (3.9)	TAS-20	None	fMRI 1.5T	10 x 10 x 10

Abbreviations: BDI: Beck Depression Inventory; BVAQ: Bermond-Vorst Alexithymia Questionnaire; fMRI: functional magnetic resonance imaging; HA: high alexithymia group; I: intermediate: LA: low alexithymia group; N: number of participants; NS: not specified; PANAS PA: Positive And Negative Affect Schedule, positive affect; PET: positron emission tomography; SD: standard deviation; STAI: State Trait Anxiety Inventory; TAS: Toronto Alexithymia Scale

Table 2.2 Overview of task paradigms applied in the included studies

Study	Faces				IAPS	Other	Valence		Task	Whole
	Sad	Angry	Fear	Happy			Pos	Neg		brain/ROI
Reker et al. (201)	x			x			x	x	Rating valence of neutral mask	Combined
Lee et al. (2011)	x	x						x	Rating arousal and valence after each session	ROI
Eichmann et al. (2008)	x			x			x	x	Rating valence of neutral mask	ROI
Kugel et al. (2008)	x			x			x	x	Rating valence of neutral mask	ROI
Kano et al. (2003)	x	x		x			x	x	Gender decision	Whole brain
Mériau et al. (2006)		x	x					x	Gender or emotional valence decision	Combined
Heinzel et al. (2010)			x	x	x		x	x	Passive viewing	Combined
Berthoz et al. (2002)					x		x	x	Passive viewing (rating valence after scan)	ROI
Bird et al. (2010)						Empathy (painful stimulation other)		x	Rating level of unpleasantness	ROI
Moriguchi et al. (2007)						Empathy (pictures of painful situations)		x	Rating pain intensity of others	Combined
Noll-Hussong et al. (2013)						Pictures of painful situations		x	Rating subjective intensity of pain	ROI
Kano et al. (2007)						Pain		x	Rating visceral sensation	Whole brain
Strigo et al. (2013)						Pain and anticipation of pain		x	Rating various variables after scan	ROI
Pouga et al. (2010)						Wathcing fearful actions		x	Oddball-task	Combined
Mantani et al. (2005)						Imagining past and future events	x	x	Rating vividness and emotional intensity	Combined

Abbreviations: IAPS: International Affective Picture System; Neg: negative; Pos: positive; ROI: region of interest

Negative stimuli

Fifteen studies were identified which examined alexithymia during the processing of negative stimuli. Of these studies, seven used a ROI approach, two a whole brain approach and six used both a whole-brain and ROI approach. Details on the included regions of interest per study can be found in supplementary Table S2.1. The total sample comprised 415 individuals (239 male and 176 female). Demographic details of the participants are shown in Table 2.1.

For negative stimuli (contrast negative>neutral/baseline) greater activation in the dorsal ACC and the right middle temporal gyrus was found in people with higher levels of alexithymia (Figure 2.2A and Table 2.3A). Furthermore, higher levels of alexithymia were associated with less activation of the left premotor cortex, bilateral fusiform gyrus, bilateral amygdala, bilateral supplementary motor area, the left dorsomedial prefrontal cortex (dMPFC), the left middle occipital gyrus, the right putamen and the left superior parietal gyrus (Figure 2.2C and Table 2.3B). Four significant clusters did not survive the extent threshold of 100 mm^3. These findings are represented in the supplementary Table S2.3.

To examine the relative contribution of the included studies to the summary findings, the distance ρ was calculated voxel-wise between the coordinates of the significant findings in the summary map and the local maxima reported by the included studies. The results revealed that the significant findings were not solely influenced by studies implementing the same task paradigms (see supplementary Table S2.4).

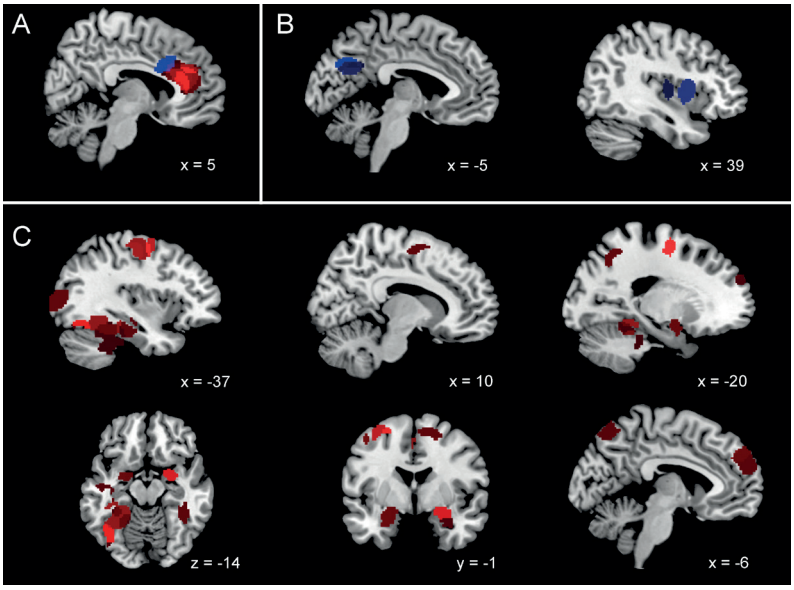
Positive stimuli

Seven studies were included that examined the neural correlates of alexithymia during the processing of positive stimuli. Of these studies, three used a ROI-based approach, one investigated the whole brain and three applied both methods (for details on used regions of interest per study, see supplementary Table S2.1). The total sample consisted of 196 participants (141 male and 55 female). Demographic details can be found in Table 2.1.

For positive stimuli (contrast positive>neutral/baseline) higher alexithymia was associated with increased activation of the dorsal ACC and middle cingulate cortex, in part overlapping the higher activity in the dorsal ACC found during negative emotional processing (Figure 2.2A and Table 2.3C). Lower activation relative to higher levels of alexithymia was found in the precuneus, cuneus, right posterior and anterior insula and in the left superior temporal gyrus (Figure 2.2B and Table 2.3D). Several studies contributed to these findings which can be found in supplementary Table S2.4. Seven significant clusters did not survive the extent threshold of 100 mm^3 (cf. supplementary Table S2.3).

|DISCUSSION

The aim of the present meta-analysis was to identify the neural correlates of alexithymia during emotion processing. Independent from valence, alexithymia was associated with higher activation in the dorsal anterior cingulate cortex (ACC) and middle cingulate cortex. In addition, valence-specific effects of alexithymia were observed, with lower activation in the amygdala, fusiform gyrus, premotor areas and the dorsomedial prefrontal cortex (dMPFC) for negative stimuli, and lower activation of the right insula and precuneus for positive stimuli. Most of the results were influenced by studies applying various task paradigms



|Figure 2.2 Brain regions with altered activation during emotion processing in alexithymia. (A) Increased activation in alexithymia during the processing of positive (bleu) and negative (red) stimuli. (B) Decreased activation in alexithymia during the processing of positive stimuli. (C) Decreased activation in alexithymia during the processing of negative stimuli. Numbers represent the sagittal (x), coronal (y) of axial (z) coordinate of each slice. Results are displayed at p<.05 FDR corrected and overlaid on a MNI template brain

suggesting that these regions show atypical activation patterns in alexithymia across a wide range of emotion processing tasks. Visual inspection of the results did not indicate a right hemisphere deficit or a left hemisphere preference in alexithymia.

Valence-independent emotional processing

The finding of increased activation in the dorsal ACC and middle cingulate cortex in alexithymia was consistent for both negative and positive stimuli. In their 'blindfeel' hypothesis, Lane et al. (1997) proposed that a dysfunction of the ACC underlies the problems in the conscious experience of emotions observed in alexithymia (Lane et al., 1997). The dorsal ACC is involved in various cognitive and emotional processes (Etkin et al., 2011) and increased activation in this area reflects higher cognitive demand during both these processes (Koch et al., 2012; Mulert et al., 2005; Taylor et al., 2003; Urry et al., 2009). In their reviews, Kano & Fukudo (2013) and Moriguchi & Komaki (2013) proposed that when stimuli have a physical context (e.g. pain stimulation), activation in several somatosensory areas (including the dorsal ACC) increases in individuals with alexithymia. They suggested that this enhanced activation might be associated with the amplification of physical sensations in individuals with alexithymia. However, only two studies contributing to the finding of increased dorsal ACC activation applied paradigms including physical stimuli (e.g. painful visceral stimulation or pictures of hands and feet in painful situations) (Kano et al., 2007; Moriguchi et al., 2007), while the other contributing studies (Berthoz et al., 2002; Heinzel et al., 2010; Mériau et al., 2006; Pouga et al., 2010) did not include stimuli with a physical context. These latter studies applied emotional picture viewing task paradigms

|Table 2.3 Summary of significant findings of neural correlates with alexithymia during negative or positive emotional processing

Cluster region	Volume (mm^3)	Anatomical label (based on AAL)	T max score	r max score	MNI coordinates x	y	z	df
(A) Positive correlations with alexithymia for the contrast negative>neutral/baseline								
Dorsal anterior cingulate cortex	15144	R Anterior cingulate gyrus	21.64	.590	2	40	10	9
		L Anterior cingulate gyrus	19.93	.567	-2	34	12	9
		No AAL label	16.70	.567	0	30	8	7
		L Superior frontal gyrus	10.13	.533	0	46	20	8
		R Middle cingulate gyrus	8.77	.523	14	28	32	8
		L Middle cingulate gyrus	4.20	.469	0	24	34	8
R Middle temporal gyrus	128	R Middle temporal gyrus	4.58	.498	60	-42	0	7
(B) Negative correlations with alexithymia for the contrast negative>neutral/baseline								
L Premotor cortex	5320	No AAL label	-17.65	-.515	-26	-8	46	7
		L Precentral gyrus	-16.60	-.515	-26	-8	48	8
		L Superior frontal gyrus	-16.44	-.511	-24	-10	52	8
		L Middle frontal gyrus	-16.44	-.511	-26	-8	50	8
L Fusiform gyrus	18024	L Fusiform gyrus	-14.21	-.471	-42	-58	-22	8
		L Fusiform gyrus	-9.36	-.461	-36	-30	-12	7
		L Interior temporal gyrus	-9.36	-.461	-44	-36	-18	7
		L Cerebellum	-8.42	-.517	-30	-40	-26	7
		L Cerebellum	-8.42	-.517	-30	-40	-28	7
		L Hippocampus	-5.61	-.463	-36	-16	-16	7
		L Middle temporal gyrus	-5.61	-.463	-46	-10	-20	7
		L Lingual gyrus	-4.71	-.473	-22	-54	-12	7
		L Cerebellum	-4.43	-.464	-36	-42	-38	7
		L Cerebellum	-4.43	-.464	-38	-38	-40	7
		L Cerebellum	-4.43	-.464	-34	-44	-42	7
		L Cerebellum	-4.43	-.464	-26	-40	-40	7
R Amygdala	1944	R Amygdala	-12.48	-.410	24	0	-20	9
L Suppl motor area	176	L Suppl motor area	-8.23	-.473	-2	0	-20	8
		L Middle cingulate gyrus	-8.22	-.475	0	-2	50	8
L Dorsomedial prefrontal cortex	6488	L Medial superior frontal gyrus	-7.43	-.416	-8	44	30	8

|Table 2.3 Continued

Cluster region	Volume (mm^3)	Anatomical label (based on AAL)	T max score	r max score	MNI coordinates x	y	z	df
		L Superior frontal gyrus	-6.54	-.417	-10	56	42	8
		L Middle frontal gyrus	-5.54	-.418	-18	50	28	8
R Suppl motor area	2048	R Superior frontal gyrus	-6.77	-.458	20	-4	54	7
		R Superior frontal gyrus	-6.05	-.461	22	-4	56	8
		R Suppl motor area	-6.05	-.461	12	-4	54	8
L Amygdala	1720	L Amygdala	-6.68	-.367	-28	-2	-26	9
L Superior parietal gyrus	4752	L Superior parietal gyrus	-6.21	-.484	-24	-54	42	7
		L Superior parietal gyrus	-6.21	-.484	-20	-62	40	7
		L Inferior parietal gyrus	-6.21	-.484	-22	-50	54	7
		L Precuneus	-5.96	-.481	-12	-66	48	7
		L Middle occipital gyrus	-5.53	-.452	-22	-60	40	7
L Middle occipital gyrus	4000	No AAL label	-6.21	-.432	-40	-94	-2	7
		L Middle occipital gyrus	-6.21	-.432	-36	-94	-2	7
		L Inferior occipital gyrus	-6.21	-.432	-32	-84	0	7
R Fusiform gyrus	2216	R Fusiform gyrus	-5.97	-.440	44	-44	-22	8
L Middle occipital gyrus	224	L Middle occipital gyrus	-5.87	-.478	-24	-86	16	7
R Putamen	216	No AAL label	-5.11	-.453	32	-12	14	7
		R Putamen	-4.93	-.422	30	-10	14	8
(C) Positive correlations with alexithymia for the contrast positive>neutral/baseline								
Dorsal anterior cingulate cortex	3040	L Anterior cingulate gyrus	14.83	.516	-6	12	26	4
		L Anterior cingulate gyrus	14.33	.516	-4	12	24	4
		R Anterior cingulate gyrus	13.15	.518	2	10	26	4
		L Middle cingulate gyrus	13.15	.518	0	14	34	4
		R Middle cingulate gyrus	13.15	.518	2	10	32	4
(D) Negative correlations with alexithymia for the contrast positive>neutral/baseline								
Precuneus/Cuneus	7400	L Cuneus	-27.96	-.550	-10	-68	26	3

|Table 2.3 Continued

Cluster region	Volume (mm^3)	Anatomical label (based on the AAL)	T max score	r max score	MNI coordinates			df
					x	y	z	
		L Precuneus	-27.96	-.550	-10	-58	28	3
		R Precuneus	-21.18	-.550	4	-70	34	3
		L Calcarine	-14.42	-.546	-4	-70	22	3
		R Calcarine	-11.59	-.544	2	-62	20	3
		R Cuneus	-10.94	-.543	4	-74	24	3
		L Posterior cingulate gyrus	-9.71	-.544	-2	-52	26	4
		R Precuneus	-7.74	-.541	4	-56	32	4
		R Middle cingulate gyrus	-7.69	-.540	2	-52	34	4
L Superior temporal gyrus	256	No AAL label	-16.42	-.586	-44	-12	-16	3
		L Superior temporal gyrus	-16.42	-.586	-46	-14	-10	3
		L Middle temporal gyrus	-16.42	-.586	-46	-12	-16	3
R Anterior insula	4744	R inferior frontal gyrus	-16.26	-.577	50	10	4	4
		R Rolandic operculum	-16.26	-.577	50	4	2	4
		R Insula	-16.26	-.577	40	4	-6	4
		R Insula	-15.44	-.575	38	4	-6	3
		R Putamen	-15.44	-.575	34	4	-4	3
R Posterior insula	2016	R Insula	-10.30	-.612	36	-14	10	4
		R Rolandic operculum	-9.05	-.617	50	-2	4	4
		R Insula	-8.55	-.614	38	-10	2	3
		R Heschl's gyrus	-8.55	-.614	44	-14	6	3
		R Superior temporal gyrus	-8.55	-.614	44	-8	-4	3

Abbreviations: AAL: anatomical automatic labeling; df: degrees of freedom; L: left; R: right; Suppl: supplementary

which did not require explicit judgment of emotional valence (e.g. gender decision, oddball-task, passive viewing) but all required some form of cognitive processing and attention, including passive viewing (Pessoa et al., 2002). Given the involvement of the dorsal ACC in both cognitive and emotional processing (Etkin et al., 2011), the increase in dorsal ACC activity may be associated with increased cognitive demand in individuals with alexithymia. This hypothesis is supported by an EEG-study of Pollatos and Gramann (2011), in which alexithymia was associated with higher N2 amplitudes during emotion processing, reflecting higher levels of cognitive demand (Pollatos and Gramann, 2011). This indicates that individuals with high scores on alexithymia may call upon neural resources to a stronger extent to attend and understand the emotional stimulus.

Although the results of the current meta-analysis showed increased activation in the dorsal ACC, various studies on alexithymia reported decreased ACC activation during emotion processing. Pouga et al. (2010) and Kano and Fukudo (2013) suggested that these discrepancies regarding ACC activation may be due to differences in task paradigms and stimuli between studies. Notably, most studies reporting lower activation in the dorsal ACC during emotion processing in association with alexithymia (Kano et al., 2003; Karlsson et al., 2008; Moriguchi et al., 2007) applied tasks which require more cognitive processing of the emotional stimuli, such as rating the valence of emotional video clips (Karlsson et al., 2008) or empathizing (Moriguchi et al., 2007). If passive emotional processing already requires additional neural resources in individuals with alexithymia, tasks which require even more emotional awareness may become too strenuous. Therefore, we propose an inverted U-shape of dorsal ACC activation in alexithymia to explain the differences between ACC activation findings in alexithymia literature. Such an inverted U-shape pattern in dorsal ACC activation has previously been shown in patients with obsessive compulsive disorder (OCD) during the N-back task (Koch et al., 2012). When the task load increased from 1-back to 2-back, dorsal ACC activity increased in this group of patients compared to healthy controls (i.e. compensation). However, when the task load increased even further (3-back), dorsal ACC activation dropped and became lower in comparison to healthy controls. This inverted U-shape hypothesis might also explain the discrepancy between the current finding of increased dorsal ACC activity and the study of McRae et al. (2008) who reported that individuals with high trait emotional awareness (which is negatively associated with alexithymia) showed increased dorsal ACC activation when viewing high arousing emotional stimuli (McRae et al., 2008). Processing of high arousing stimuli requires more allocation of attention and more complex emotional processing which for individuals with alexithymia might be too demanding. However, another possible explanation for this discrepancy between our findings and the findings of McRae et al. (2008) could be that individuals with high trait emotional awareness are more focused on high arousing stimli, which is reflected in increased brain activation, while the attention of individuals with alexithymia is less directed towards these stimuli for which they have to compensate.

The current meta-analysis showed that alexithymia is associated with increased dorsal ACC activity, possibly reflecting either the amplification of physical sensations or the need to call upon neural resources to a greater extent, depending on the type of emotion processing task. Further studies are needed to assess the association between cognitive load and emotional processing in alexithymia to verify this inverted U-shape theory.

Valence-specific emotional processing

Negative emotional stimuli

The results of this meta-analysis revealed that alexithymia is associated with decreased activation in several brain regions involved in the processing of negative emotional stimuli. For example, activation in a system important for emotional attention including the amygdala, fusiform gyrus and middle occipital cortex, was lower in individuals with alexithymia. The amygdala is a structure widely known for its role in emotion processing (Phillips et al., 2003) and becomes activated when emotional stimuli are presented. Via a feedback loop through the fusiform gyrus, the amygdala influences the occipital cortex directing visual attention towards emotional stimuli (Vuilleumier, 2005). As a consequence, individuals pay more attention to emotional stimuli than to neutral stimuli (Hodsoll et al., 2011). However, when the feedback system fails (e.g. because of amygdala lesions), attention towards emotional or novel stimuli declines (Anderson and Phelps, 2001; Jacobs et al.,

2012). Such a decline in emotional attention is also seen in individuals with alexithymia as they are less distracted by negative words (Mueller et al., 2006) and are impaired in the recall of emotional distractors (Suslow et al., 2003). Suslow et al. (2003) showed that this impairment in recall was particularly related to difficulties in the identification of feelings. An fMRI-study examining the unconscious processing of surprised faces reported lower activation in the fusiform gyrus in alexithymia, also related to the difficulties in identifying feelings (Duan et al., 2010). Moreover, an association between decreased activation in the amygdala and difficulties in identifying feelings has been suggested by several other reviews as well (Moriguchi and Komaki, 2013; Wingbermühle et al., 2012). Therefore, the lower activation found in the amygdala, fusiform gyrus and occipital cortex may be related to reduced emotional attention in individuals with alexithymia, which might underlie their difficulties in identifying emotions. This finding might seem counterintuitive with the finding of increased dorsal ACC activity as discussed above. However, if attention is less automatically directed towards emotional stimuli by a decrease in activation of the amygdala, fusiform gyrus and occipital cortex, tasks in which individuals are explicitly instructed to keep their attention towards emotional stimuli might require activation from a region involved in top-down attention, such as the dorsal ACC, to a stronger extent.

Besides the above mentioned regions, lower activation in the dorsal premotor cortex, the superior/inferior parietal cortex and supplementary motor area was found in alexithymia during the processing of negative emotional stimuli. Although not part of the classic mirror neuron system (MNS) (Rizzolatti and Craighero, 2004), these areas appear to have mirror neuron properties (Gazzola and Keysers, 2009; Molenberghs et al., 2012; Mukamel et al., 2010). This means that these areas are activated during the observation, imitation and execution of motor actions (Molenberghs et al., 2009), but also of emotional facial expressions (Carr et al., 2003). Therefore, these regions are important during emotion processing and empathy (Lamm et al., 2011), the ability to understand other people's feelings, which is impaired in individuals with alexithymia (Grynberg et al., 2010; Guttman and Laporte, 2002). Poor empathic skills in individuals with alexithymia might be related to the decreased activation in brain areas with mirror properties found in this meta-analysis. In a study directly investigating the MNS in alexithymia, individuals with and without alexithymia were presented with a classic MNS task (observation and execution of motor actions). Individuals in the high alexithymia group showed increased activation in the premotor cortex and the superior/inferior parietal cortex in comparison to the low alexithymia group, while performing equally well on the MNS task (Moriguchi et al., 2009). According to the authors, the increased neural response represented a compensation mechanism. Furthermore, they suggested that this compensation mechanism might fail during empathy and general emotion processing in alexithymia. The results of this meta-analysis are in line with this interpretation and suggest that decreased activation in the dorsal premotor cortex, superior/inferior parietal cortex and supplementary motor areas identified may be related to poorer empathic abilities in alexithymia.

The dMPFC, found to be less activated in alexithymia, is involved in various cognitive emotional processes, including social cognition and emotion regulation (Amodio and Frith, 2006; Ochsner and Gross, 2005). Lower activation in the dMPFC in alexithymia has previously been shown during a social cognition task (Moriguchi et al., 2006). Furthermore, an EEG-study on emotion regulation in alexithymia (Pollatos and Gramann, 2012) reported reduced P3 and slow wave amplitudes in low alexithymic individuals during reappraisal, but not in high alexithymic individuals, indicating that the top-down regulation in individuals with alexithymia is compromised. Although emotion regulation was not investigated explicitly in the studies included in this meta-analysis, it is conceivable that implicit regulation takes

place when individuals are presented with negative stimuli (Gyurak et al., 2011). Impairments in emotion regulation are thought to be one of the core deficits in alexithymia. For example, individuals with alexithymia report difficulties with emotion regulation (Venta et al., 2013) and make less use of cognitive reappraisal (Swart et al., 2009), the reinterpretation of emotional stimuli in such a way that it reduces their emotional impact (John and Gross, 2004). During reappraisal, prefrontal areas including the dMPFC become activated (Diekhof et al., 2011) and down-regulate activation in limbic regions (Ochsner and Gross, 2005). Indeed, reappraisal success has been found to correlate with activation in the dMPFC (Wager et al., 2008). Hence, reduced activation in this area might lead to less successful emotion regulation in alexithymia, resulting in the experience of more negative emotions (De Gucht et al., 2004; Yelsma, 2007). However, one might argue that individuals with alexithymia do not need to down-regulate amygdala activation, since activation in this area is already low in response to negative emotional stimuli (see results). Vorst and Bermond (2001) divided the alexithymia construct into a cognitive and affective dimension (Vorst and Bermond, 2001). The cognitive dimension refers to the processing of emotions at a cognitive level, such as identifying, analyzing and verbalizing emotions. In contrast, the affective dimension refers to the level of subjective emotional experience. It might be that individuals with high emotional experience but low cognitive processing capacities are unable to down-regulate their negative affect through reappraisal, resulting in an even higher experience of negative emotions. In contrast, individuals with both low emotional experience and low cognitive emotional processing capacities might not need to down-regulate their emotions, since emotional experience is already low. Unfortunately, due to the low number of neuroimaging studies examining the affective dimension, the variation on this dimension could not be taken into account in the current meta-analysis. Future research is needed to investigate the neural correlates of the two alexithymia dimensions, especially during emotion regulation, to examine this hypothesis and the role of the dMPFC in alexithymia.

Positive emotional stimuli

The results of the analysis on emotion processing of positive stimuli in alexithymia should be considered preliminary due to the low number of included whole brain studies. Nonetheless, the results revealed lower activation in the right anterior and posterior insula and the precuneus. The insula, especially the right, is considered to be a neuroanatomical substrate for emotional awareness (Critchley et al., 2004). It has been shown that the right anterior insula is functionally connected to the precuneus during the evaluation of emotional state (Terasawa et al., 2013). According to the authors, emotional awareness arises from transforming interoceptive information (insula) into a subjective emotional state (precuneus). Several other studies have underlined the involvement of the insula and precuneus in emotional awareness (for review see Tsuchiya and Adolphs, 2007). This holds for the experience of both positive and negative emotions (Craig, 2009; Habel et al., 2005). Therefore, decreased activation in these areas in alexithymia suggests reduced emotional awareness of positive affect. Bagby and Parker (1997) described individuals with alexithymia to have “a limited capacity to experience positive emotions, such as joy, happiness and love”. Since then, several studies have reported an association between alexithymia and lower positive affect (De Gucht et al., 2004; Yelsma, 2007) or a lower tendency to experience positive emotions (Luminet et al., 1999), while negative affect is increased (De Gucht et al., 2004; Yelsma, 2007). Therefore, reduced emotional awareness during positive emotion processing might underlie the lower positive affect that individuals with alexithymia experience.

Methodological issues and limitations

The studies included in this meta-analysis differed in the methods that were applied. Although part of these differences, such as sample size, smoothing kernel and statistical threshold are accounted for by the PCM method (Costafreda, 2012), differences in task paradigms, included covariates and variation in alexithymia scores introduce unavoidable heterogeneity which might have reduced the sensitivity. Furthermore, the modest number of studies included in the meta-analysis, especially regarding the positive stimuli, might have limited the statistical power to detect more subtle effects of alexithymia on brain activation during emotion processing. Therefore, the results regarding the positive stimuli should be considered preliminary and no definite conclusions can be drawn on brain regions which did not reach significance.

By applying the new parametric coordinate-based meta-analysis (PCM) method (Costafreda, 2012), we were able to include studies with a ROI approach as well as studies with a whole brain approach. Including ROI studies can provide valuable information and increases sensitivity for certain brain areas (Groenewold et al., 2012). However, by including ROI-studies, the power for certain brain areas increases, which might slightly increase the chance of significant findings in these areas in comparison to areas which are not examined with a ROI approach. In addition, it should be acknowledged that strategies of ROI selection may differ between studies.

One hypothesis regarding the lateralization of emotion processing in alexithymia could not be statistically examined in the current meta-analysis. Future studies should examine a hemisphere by task interaction in alexithymia to gain more insight into the right hemisphere deficit or left hemisphere preference in alexithymia.

Another limitation of the current meta-analysis is that only brain regions associated with cognitive alexithymia were examined. Recently, studies have indicated that the cognitive and affective dimension of alexithymia might have different underlying neural correlates (Goerlich et al., 2012; Larsen et al., 2003; Pouga et al., 2010). Unfortunately, this could not be assessed in the current meta-analysis, since only one included study examined the neural correlates of the affective alexithymia dimension (Pouga et al., 2010). Because the two different dimensions have been hypothesized to underlie different pathologies (Moormann et al., 2008a), further research on the neural correlates of these separate dimensions might give valuable insights into alexithymia and the development of psychopathology.

All studies included in the current meta-analysis assessed alexithymia through self-report measures. These measurements rely on reflecting one's own emotions which is limited in individuals with high scores on alexithymia. Therefore, several authors proposed to examine alexithymia through observer-based questionnaires (Lundh et al., 2002; Suslow et al., 2001). Unfortunately, only one study (Moriguchi et al., 2007) included an observer rated measurement to confirm the presence of alexithymia. This limits our results to self-reported alexithymia. We encourage future studies to combine self-reports with observer-rated measurements to get a more comprehensive measure of alexithymia.

Furthermore, alexithymia is related to feelings of depression and anxiety (Hendryx et al., 1991). Unfortunately, only half of the studies included in the current meta-analysis controlled for the effects of depression and anxiety (by including these factors as a covariate or by excluding individuals with high scores on anxiety and/or depression). Therefore, it is not possible to completely eliminate the effects of these mood states on the current findings. Future research should further examine the relationship between alexithymia, depression and anxiety and the effect of this relationship on brain activation.

As pointed out in the introduction, recent studies strongly suggest to conceptualize alexithymia as a dimensional construct. Most studies included in this meta-analysis adopted this view and defined alexithymia as a continuous personality dimension. However, cut-off scores for both the TAS-20 (≥ 61, Bagby et al., 1994) and the BVAQ-B (≥ 53, Deborde et al., 2008) have been formed to indicate clinical meaningful levels of alexithymia. Only a few studies included in this meta-analysis (Kano et al., 2003; Kano et al., 2007; Moriguchi et al., 2007) included a substantial number of individuals reaching the clinically relevant alexithymia scores. Therefore, it remains an open question whether the currently identified neural correlates generalize to clinical alexithymia.

|CONCLUSION

This meta-analysis identified several neural correlates of alexithymia. Specifically, in alexithymia the amygdala, a key node of the emotional perception/attention system, is less activated during negative emotional processing. While performing simple emotional processing tasks, such a deficit might be compensated by higher activation in the dorsal anterior and middle cingulate cortex. However, this compensation mechanism might fail when task difficulty increases (i.e. when individuals with alexithymia show performance reductions). When presented with physical emotional stimuli, increased activation in the dorsal anterior cingulate cortex might reflect hypersensitivity towards the physical information of these stimuli. Furthermore, lower activation was found in MNS related brain regions and the dorsomedial prefrontal cortex during the processing of negative emotional stimuli. This may underlie the impaired empathic capabilities and the difficulties in emotion regulation observed in alexithymic individuals. A decrease in insula and precuneus activation was identified during the processing of positive stimuli, possibly reflecting reduced emotional awareness, which may help explain the lower positive affect experienced by these individuals.

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|SUPPLEMENTARY MATERIAL

|Table S2.1 Details on included regions of interest per study

Study	Region of Interest (ROI)	Contrast	AAL label
Lee et al. (2011)	Amygdala	Neg>Neu negative	Amygdala_L 4201[b]
			Amygdala_R 4202[b]
	Rostral anterior cingulate	Neg>Neu negative	Cingulum_Ant L 4001[c]
			Cingulum_Ant R 4002[c]
	Frontal-striatal pathway	Neg>Neu negative	Caudate_L 7001[b]
			Caudate_R 7002*[a]
			Putamen_L 7011[b]
			Putamen_R 7012[b]
			Frontal_Med_Orb_L 2611[b]
			Frontal_Med_Orb_ R2612[b]
Heinzel et al. (2010)	Amygdala	Neg>Neu negative, postive	Amygdala_L 4201[b] Amygdala_R 4202[b]
		Pos>Neu negative, positive	Amygdala_L 4201[b] Amygdala_R 4202[b]
	Anterior cingulate	Neg>Neu positive	Cingulum_Ant_L 4001*[a]
			Cingulum_Ant_R 4002*[a]
		Pos>Neu positive	Cingulum_Ant_L 4001[b]
			Cingulum_Ant_R 4002*[a]
Bird et al. (2010)	Left anterior insula	Neg>Neu negative	Insula_L 3001*[a]
Pouga et al. (2010)	Inferior frontal gyrus	Neg>Neu negative, positive	Frontal_Inf_Tri_L 2311[a] Frontal_Inf_Tri_R 2312[a]
			Frontal_Inf_Oper_L 2301[a]
			Frontal_Inf_Oper_R 2302[a]
	Superior temporal sulcus	Neg>Neu, negative, positive	Temporal_Sup_L 8111[a] Temporal_Sup_R 8112[a]
	Left postcentral gyrus	Neg>Neu, negative, positive	Postcentral_L 6001[a]
	Right precentral gyrus	Neg>Neu postive	Precentral_R 2002[a]

|Table S2.1 Continued

Study	Region of interest (ROI)	Contrast	AAL label
	RIght temporal pole	Neg>Neu negative, positive	Temporal_Pole_Sup_R 8122[a]
			Temporal_Pole_Mid_R 8212[a]
	Amygdala	Neg>Neu positive	Amygdala_R 4202[a]
			Amygdala_L 4201[a]
		Neg>Neu negative	Amygdala_R 4202*[a]
			Amygdala_L 4201*[a]
	Right anterior cingulate	Neg>Neu negative	Cingulum_Ant_R 4002[a]
Reker et al. (2010)	Amygdala	Pos>Neu negative, positive	Amygdala_L 4201[c] Amygdala_R 4202[c]
		Neg>Neu negative	Amygdala_L 4201*[a]
			Amygdala_R 4202[c]
		Neg>Neu positive	Amygdala_L 4201[c]
			Amygdala_R 4202[c]
	Left cuneus	Pos>Neu negative	Cuneus_L 5011*[a]
	Middle occipital gyrus	Pos>Neu negative	Occipital_Mid_L 5201*[a]
		Neg>Neu negative	Occipital_Mid_L 5201*[a]
	Fusiform gyrus	Pos>Neu negative	Fusiform_L 5401[c]
			Fusiform_R 5402[c]
		Pos>Neu positive	Fusiform_L 5401[c]
			Fusiform_R 5402*[a]
		Neg>Neu negative	Fusiform_L 5401*[a]
			Fusiform_R 5402[c]
		Neg>Neu positive	Fusiform_L 5401[c]
			Fusiform_R 5402[c]
	Superior temporal gyrus	Pos>Neu negative	Temporal_Sup_L 8111*[a]
			Temporal_Sup_R 8112*[a]
		Pos>Neu positive	Temporal_Sup_L 8111[c]
			Temporal_Sup_R 8112[c]
		Neg>Neu negative	Temporal_Sup_L 8111*[a]
			Temporal_Sup_R 8112[c]
		Neg>Neu positive	Temporal_Sup_L 8111[c]
			Temporal_Sup_R 8112[c]
	Insula	Pos>Neu negative	Insula_L 3001*[a]

|Table S2.1 Continued

Study	Region of interest (ROI)	Contrast	AAL label
			Insula_R 3002*[a]
		Pos>Neu positive	Insula_L 3001[c]
			Insula_R 3002[c]
		Neg>Neu negative	Insula_L 3001*[a]
			Insula_R 3002*[a]
		Neg>Neu positive	Insula_L 3001[c]
			Insula_R 3002[c]
	Postcentral gyrus	Pos>Neu negative	Postcentral_L 6001*[a]
			Postcentral_R 6002[c]
		Pos>Neu positive	Postcentral_L 6001[c]
			Postcentral_R 6002[c]
		Neg>Neu negative, positive	Postcentral_L 6001[c] Postcentral_R 6002[c]
	Right Angular gyrus	Pos>Neu negative	Angular_R 6222*[a]
	Right Precuneus	Pos>Neu negative	Precuneus_R 6302*[a]
	Orbitofrontal gyrus	Pos>Neu negative, positive	Frontal_Sup_Orb_L 2111[c]
			Frontal_Sup_Orb_R 2112[c]
			Frontal_Mid_Orb_L 2211[c]
			Frontal_Mid_Orb_R 2212[c]
			Frontal_Inf_Orb_L 2321[c]
			Frontal_Inf_Orb_R 2322[c]
		Neg>Neu negative, positive	Frontal_Sup_Orb_L 2111[c]
			Frontal_Sup_Orb_R 2112[c]
			Frontal_Mid_Orb_L 2211[c]
			Frontal_Mid_Orb_R 2212[c]
			Frontal_Inf_Orb_L 2321[c]
	Inferior frontal gyrus	Pos>Neu negative	Frontal_Inf_Oper_L 2301*[a]

|Table S2.1 Continued

Study	Region of interest (ROI)	Contrast	AAL label
			Frontal_Inf_Oper_R 2302[c]
			Frontal_Inf_Tri_L 2311[c]
			Frontal_Inf_Tri_R 2312[c]
		Pos>Neu positive	Frontal_Inf_Oper_L 2301[c]
			Frontal_Inf_Oper_R 2302[c]
			Frontal_Inf_Tri_L 2311[c]
			Frontal_Inf_Tri_R 2312[c]
		Neg>Neu negative, positive	Frontal_Inf_Oper_L 2301[c]
			Frontal_Inf_Oper_R 2302[c]
			Frontal_Inf_Tri_L 2311[c]
			Frontal_Inf_Tri_R 2312[c]
	Rolandic Operculum	Pos>Neu positive	Rolandic_Oper_L 2331[c]
			Rolandic_Oper_R 2332[c]
		Neg>Neu negative, positive	Rolandic_Oper_L 2331[c] Rolandic_Oper_R 2332[c]
	Left Parahippocampal gyrus	Neg>Neu negative	ParaHippocampal_L 4111[*a]
Eichmann et al. (2008)	Fusiform gyrus	Neg>Neu negative	Fusiform_5401[*b]
			Fusiform_R 5402[*b]
		Neg>Neu positive	Fusiform_L 5401[b]
			Fusiform_R 5402[b]
		Pos>Neu negative, positive	Fusiform_L 5401[b] Fusiform_R 5402[b]
Kugel et al. (2008)		Neg>Neu negative	Amygdala_L 4201[*b]
			Amygdala_R 4202[*b]
		Neg>Neu positive	Amygdala_L 4201[b]
			Amygdala_R 4202[b]
		Pos>Neu negative, positive	Amygdala_L 4201[b] Amygdala_R 4202[b]

|Table S2.1 Continued

Study	Region of interest (ROI)	Contrast	AAL label
Moriguchi et al. (2007)	Postcentral gyrus	Neg>Neu negative, positive	Postcentral_L 6001[c] Postcentral_R 6002[c]
	Rolandic Operculum	Neg>Neu negative, positive	Rolandic_Oper_L 2331[c] Rolandic_Oper_R 2332[c]
	Insula	Neg>Neu negative, positive	Insula_L 3001[c] Insula_R 3002[c]
	Caudal Anterior Cingulate	Neg>Neu negative	Cingulum_Ant_L 4001[*a] Cingulum_Ant_R 4002[c]
		Neg>Neu positive	Cingulum_Ant_L 4001[c]
			Cingulum_Ant_R 4002[c]
	Thalamus	Neg>Neu negative, positive	Thalamus_L 7101[c] Thalamus_R 7102[c]
	Lateral prefrontal cortex	Neg>Neu negative, positive	Frontal_Mid_Orb_L 2211[c]
			Frontal_Mid_Orb_R 2212[c]
			Frontal_Inf_Orb_L 2321[c]
			Frontal_Inf_Orb_R 2322[c]
			Frontal_Mid_L 2201[c]
			Frontal_Mid_R 2202[c]
Mantani et al. (2005)	Medial parieto-occipital area	Pos>Neu negative, positive	Cingulum_Post_L 4021[*a,c]
			Cingulum_Post_R 4022[*a,c]
		Neg>Neu negative, positive	Cingulum_Post_L 4021[*a,c]
			Cingulum_Post_R 4022[*a,c]
		Pos>Neu negative, positive	Cuneus_L 5011[c] Cuneus_R 5012[c]
		Neg>Neu negative, positive	Cuneus_L 5011[c] Cuneus_R 5012[c]
		Pos>Neu negative	Precuneus_L 6301[c]
			Precuneus_R 6302[c]

|Table S2.1 Continued

Study	Region of interest (ROI)	Contrast	AAL label
		Neg>Neu negative, postive	Precuneus_L 6301[c]
			Precuneus_R 6302[c]
	Anterior Cingulate	Pos>Neu negative	Cingulum_Ant_L 4001[c]
			Cingulum_Ant_R 4002[c]
	Superior Medial frontal gyrus	Pos>Neu negative	Frontal_Sup_Medial_L 2601[c]
			Frontal_Sup_Medial_R 2602[c]
Berthoz et al. (2002)	Left Superior Medial frontal	Neg>Neu negative	Frontal_Sup_Medial_L 2601*[a]
	Anterior Cingulate	Pos>Neu positive	Cingulum_Ant_L 4001*[a]
			Cingulum_Ant_R 4002*[a]
	Medial Prefrontal cortex	Pos>Neu positive	Frontal_Mid_L 2201*[a]
			Frontal_Mid_R 2202*[a]
			Frontal_Sup_L 2101*[a]
			Frontal_Inf_Tri_R 2312*[a]
	Middle Cingulate Gyrus	Pos>Neu positive	Cingulum_Mid_L 4011*[a]
			Cingulum_Mid_R4012*[a]
Mériau et al. (2006)	Anterior Cingulate Gyrus	Neg>Neu positive	Cingulum_Ant_L 4001[c]
			Cingulum_Ant_R 4002*[c]
Noll-Hussong et al. (2013)	Insula	Neg>Neu negative, positive	Insula_L 3001[b] Insula_R 3002[b]
Strigo et al. (2013)	Right anterior insula	Neg>Neu negative, positive	Insula_R 3002[c]
	Right posterior insula	Neg>Neu negative, positive	Insula_R 3002[c]

* significant finding; [a] coordinates were given in the original paper; [b] AAL label as given in the original paper; [c] AAL label as given by the authors of this meta-analysis in consultation with a trained anatomist. *Abbreviations*: AAL: anatomical automatic labeling; Neg: negative; Negative: negative correlation; Neu: neutral; Pos: positive; Positive: positive correlation

|Table S2.2 Reasons for exclusion of studies examining alexithymia-related brain activation

Study	Task	Reason for exclusion
Liemburg et al. (2012)	Resting state	No emotion processing task
Miyake et al. (2012)	Processing of negative words	Alexithymia-related brain activation only assessed in patients
De Greck et al. (2012)	Reward anticipation task Empathy task	Alexithymia-related brain activation only assessed in patients and healthy controls combined
Koelkebeck et al. (2011)	Theory of Mind task	No emotion processing task
Zotev et al. (2011)	Neurofeedback task	No emotion processing task
Kano et al. (2011)	Gambling task	Unsuitable contrast (positive vs negative)
Swart et al. (2011)	Valence evaluation of words	Unsuitable contrast (pos and neg vs non-emotional)
Payer et al. (2011)	Affect matching and labeling	Unsuitable contrast (pos and neg vs non-emotional)
Heinzel et al. (2010)	Explicit emotional processing task	Unsuitable contrast (correlation between alexithymia and valence scores from negative to positive)
Duan et al. (2010)	Implicit emotional processing	Unsuitable contrast (surprised vs neutral)
Miyake et al. (2009)	Emotional decision making task	No healthy controls
Moriguchi et al. (2009)	Mirror Neuron System task	No emotion processing task
Silani et al. (2008)	Explicit emotion processing task	Unsuitable contrast (external vs internal processing)
Frewen et al. (2008)	Trauma-script Imagery	No healthy controls
Karlsson et al. (2008)	Watching emotional film clips	Unsuitable contrast (pos and neg vs non-emotional)
Frewen et al. (2006)	Trauma-script Imagery	Unclear methods/results
Moriguchi et al. (2006)	Theory of Mind task	No emotion processing task
Li et al. (2006)	Script-guided imagery	No healthy controls
Sutherland et al. (2013)	Resting state	No emotion processing task

Abbreviations: neg: negative; pos: positive; vs: versus

References of Table S2.2

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In the main article, results were only considered significant when the clusters of voxels were larger than the chosen extent threshold of 100 mm^3. This threshold is applied because it is generally accepted that "small" clusters of activation may represent spurious findings. However, the size of such an extent threshold is an arbitrary decision. Therefore, Table S2.3 represents the clusters found for each analysis below the 100 mm^3 threshold for completeness.

|Table S2.3 Summary of significant findings of neural correlates associated with alexithymia during negative or positive emotional processing below the 100 mm^3 cluster extent threshold

Cluster region	Volume (mm^3)	Anatomical label (based on AAL)	T max score	r max score	MNI coordinates x	y	z	df
(A) Positive correlations with alexithymia for the contrast Negative > Neutral/Baseline								
R Caudate	32	R Caudate	4.73	.456	8	12	16	8
R Caudate	32	R Caudate	4.67	.455	12	14	18	9
(B) Negative correlations with alexithymia for the contrast Negative > Neutral/Baseline								
L Heschl's gyrus	64	L Heschl's gyrus	-6.00	-.470	-42	-16	4	8
L Cerebellum	72	No AAL label	-4.37	-.464	-22	-46	-34	8
(C) Positive correlations with alexithymia for the contrast Positive > Neutral/Baseline								
R Caudate	8	R Caudate	11.72	.519	18	16	18	5
R Caudate	24	R Caudate	11.72	.519	16	12	20	5
R Caudate	8	R Caudate	11.12	.519	14	10	22	5
(D) Negative correlations with alexithymia for the contrast Positive > Neutral/Baseline								
L Thalamus	16	L Thalamus	-13.20	-.597	-20	-24	12	4
R Putamen	8	No AAL Label	-11.22	-.604	32	-12	12	4
R Insula	8	No AAL Label	-11.22	-.604	34	-12	14	4
L Cuneus	16	L Cuneus	-10.56	-.591	-8	-80	20	4

Abbreviations: AAL: anatomical automatic labeling; df: degrees of freedom; L: left; R: right

To examine the contribution of the included studies to the summary findings, the distance ρ was calculated voxel-wise between the coordinates of the significant findings in the summary map and the local maxima reported by the included studies. A coordinate influenced a summary finding when the distance was less than 2 * radius (2 * 15 mm = 30 mm).

|Table S2.4 Studies which contributed to the significant findings

Significant findings	Studies contributing to this cluster
(A) Positive correlations with alexithymia for the contrast Negative > Neutral/Baseline	
Dorsal anterior cingulate cortex	Heinzel et al., 2010
	Pouga et al., 2010
	Kano et al., 2007
	Moriguchi et al., 2007
	Mériau et al., 2006
(B) Negative correlations with alexithymia for the contrast Negative > Neutral/Baseline	
Amygdala, Fusiform gyrus, Occipital cortex	Reker et al., 2010
	Kano et al., 2007
	Moriguchi et al., 2007
	Kano et al., 2003
	Eichmann et al., 2008
	Pouga et al., 2010
	Kugel et al., 2008
Premotor cortex, Suppl motor area, Parietal gyrus	Kano et al., 2003
	Kano et al., 2007
	Pouga et al., 2010
	Moriguchi et al., 2007
Dorsal medial prefrontal cortex	Moriguchi et al., 2007
	Berthoz et al., 2002
(C) Positive correlations with alexithymia for the contrast Positive > Neutral/Baseline	
Dorsal anterior cingulate cortex	Heinzel et al., 2010
	Berthoz et al., 2002

|Table S2.4 Continued

Significant findings	Studies contributing to this cluster
(D) Negative correlations with alexithymia for the contrast Negative > Neutral/Baseline	
Precuneus, Cuneus, Insula, Superior temporal gyrus	Reker et al., 2010
	Kano et al., 2003
	Mantani et al., 2005

Abbreviations: Suppl: supplementary

3 Dissociale morphometric profiles of the affective and cognitive dimensions of alexithymia

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Cortex, 2014; 54: 190-199.

|ABSTRACT

BACKGROUND: Alexithymia ("no words for feelings") is a psychological construct that can be divided in a cognitive and affective dimension. The cognitive dimension reflects the ability to identify, verbalize and analyze feelings, whereas the affective dimension reflects the degree to which individuals get aroused by emotional stimuli and their ability to fantasize. These two alexithymia dimensions may differentially put individuals at risk to develop psychopathology. However, their neural correlates have rarely been investigated. The aim of the current study was to investigate whether the cognitive and affective alexithymia dimension are associated with unique anatomical profiles.

METHODS: Structural MRI scans of 57 participants (29 males; mean age: 34) were processed using a voxel-based morphometry (VBM) -Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) approach. Multiple regression analyses were performed to examine the common and specific associations between gray and white matter volume and alexithymia subdimensions.

RESULTS: The results revealed that the cognitive dimension was related to lower dorsal anterior cingulate volume. In contrast, the affective alexithymia dimension was associated with lower gray matter volume in the medial orbitofrontal cortex and lower white matter volume in the superior longitudinal fasciculus near the angular gyrus. No relationship between corpus callosum volume and alexithymia was observed.

CONCLUSION: These results are consistent with the idea that there are two separable neural systems underlying alexithymia. This finding might encourage future research into the link between specific alexithymia subtypes and the development of psychopathology.

|INTRODUCTION

With a prevalence rate of ten percent in the general population (Salminen et al., 1999), alexithymia ("no words for feelings") is considered a risk factor for a range of neurological and psychiatric disorders (Taylor et al., 1997). Alexithymia is a dimensional psychological construct that is characterized by difficulties identifying and describing one's feelings as well as a difficulty in distinguishing them from bodily sensations of arousal. Alexithymia has further been associated with a lack of imagination and an externally oriented thinking style with reduced capacities of introspection (Sifneos, 1973; Vorst and Bermond, 2001).

However, it is suggested that alexithymia is not a uniform construct, but may instead comprise of an affective and a cognitive dimension (Vorst and Bermond, 2001). The affective dimension refers to the level of subjective emotional experience and consists of the factors emotionalizing (the degree to which someone is emotionally aroused by emotion-inducing events) and fantasizing (the degree to which someone is inclined to imagine, day-dream etc.). The cognitive dimension refers to the processing of emotions at a cognitive level and consists of the factors identifying, analyzing and verbalizing feelings. Based on these dimensions, Bermond et al. (2007) proposed to distinguish subtypes of alexithymia which seem to be differentially associated with psychopathology. Type-I alexithymia is characterized by high scores on both the affective and the cognitive dimension (i.e. both emotional experience and the cognitions accompanying the emotions are impaired) and has been suggested to relate to schizoid personality disorder and psychopathy (Moormann et al., 2008a). Type-II alexithymia is characterized by intact or even heightened levels of emotional experience, while cognitive emotion processing is reduced, and is associated with Borderline Personality disorder and schizophrenia (Moormann et al., 2008a; van der Meer et al., 2009). Thus, the two alexithymia dimensions might put individuals at risk for developing psychopathological disorders in different ways.

Brain regions that are thought to underlie alexithymia include both (sub)cortical regions and white matter tracts. One of the oldest theories regarding the anatomical correlates of alexithymia suggests that abnormal corpus callosum morphometry may hamper interhemispheric communication subserving cognitive processing of emotions (Gazzaniga and LeDoux, 1978), thereby contributing to the cognitive dimension (Larsen et al., 2003) and type-II alexithymia (Houtveen et al., 1997). Besides the corpus callosum, several gray matter regions are thought to be related to alexithymia. It has been proposed that dysfunction of the anterior cingulate cortex (ACC) relates to both cognitive and emotional aspects of alexithymia (Bermond et al., 2006; Lane et al., 1997; Larsen et al., 2003; Wingbermühle et al., 2012) given its involvement in emotional experience (Milad et al., 2007) and cognitive demanding emotional tasks (Phan et al., 2002). However, results relating ACC volume to alexithymia are ambiguous. For example, positive correlations between alexithymia and ACC surface have been reported (Gündel et al., 2004), while others reported lower volume in this area (Borsci et al., 2009; Ihme et al., 2013; Koven et al., 2011; Paradiso et al., 2008; Sturm and Levenson, 2011) or were unable to find a significant association (Heinzel et al., 2012). Furthermore, previous studies associated alexithymia with lower gray matter in the orbitofrontal cortex (OFC), insula, and amygdala (Borsci et al., 2009; Ihme et al., 2013), whereas another study reported increased volume in the insula associated with alexithymia (Zhang et al., 2011). These regions are involved in primary emotion identification and in the generation of emotional states (Adolphs, 2002a; Phillips et al., 2003; Vuilleumier, 2005) and are thought to underlie both the affective and cognitive alexithymia dimension (Wingbermühle et al., 2012). However, it has also been proposed that dysfunctioning of specifically the medial OFC would be associated with solely the affective dimension

(Bermond et al., 2006).

As reported above, previous studies on the structural correlates of alexithymia have produced equivocal results. One explanation for this might be that all these studies used the Toronto Alexithymia Scale (TAS-20), which assesses the cognitive alexithymia dimension only. As previously suggested by Koven et al. (2011), assessing specific dimensions of emotional constructs, including alexithymia, instead of examining it as a unitary construct, can provide a more nuanced view and can indicate whether there are separate neural correlates underlying different alexithymia dimensions. Furthermore, a recent study revealed that the two alexithymia dimensions may indeed be related to different gray matter (GM) volumes (Goerlich-Dobre et al., 2013). It was shown that cognitive alexithymia, as examined by the TAS-20, might be more associated with larger insula volume, while affective alexithymia seemed to be related to larger cingulate volume. This together with the suggestions that the affective alexithymia dimension may differentially affect the processing of emotions and seems to be related to separate neural correlates (Bermond et al., 2010; Goerlich et al., 2012; Moormann et al., 2008b; Pouga et al., 2010), indicates that the lack of controlling for scores on the affective dimension may have confounded previous findings. Furthermore, the impact of this alexithymia dimension on brain morphology, especially white matter (WM), remains unknown.

The aim of the present study was to examine whether the affective and cognitive dimensions of alexithymia are associated with different anatomical profiles. To this extent, gray and white matter volume in relation to the affective and cognitive dimensions of alexithymia was examined using voxel-based morphometry (VBM). Based on previous literature, we hypothesized that corpus callosum volume would be specifically related to the cognitive alexithymia dimension, whereas medial OFC volume was thought to be related to solely the affective alexithymia dimension. Furthermore, we predicted to find structural differences in the ACC, insula and amygdala associated with both the alexithymia dimensions.

|METHODS

Participants

Structural T1-weighted MRI scans of 60 right-handed healthy subjects (30 male and 30 female matched for age and education level, age range 18-55) were selected from one previous neuroimaging study (van der Meer et al., 2013) and two ongoing neuroimaging studies of our group[1,2]. From these previous studies, 86 unique T1-weighted MRI-scans from healthy controls (excluding siblings of patients with schizophrenia and subjects at ultra-high risk for schizophrenia) were available from studies with identical scan parameters in which the BVAQ was administered. Of these 86, 26 scans were excluded based on the following exclusion criteria: 1) older than 55 (n=2), 2) left-handed (n=15), 3) subject did not finish high school (n=1), 4) reports of present or past psychiatric or neurological problems (n=6), and 5) incomplete BVAQ (n=2). All participants gave written informed consent and the studies were approved by the local medical ethical committee. Demographic characteristics are presented in Table 3.1.

Behavioral measurements

Bermond-Vorst Alexithymia Questionnaire

The Bermond-Vorst Alexithymia Scale (BVAQ) is a 40-item self-report scale used to assess

[1] Brain activation during information and emotion processing in non-psychotic relatives of patients with schizophrenia. A multicenter study (add-on study of the national GROUP (Genetic Risk and Outcome of Psychosis) project (Korver et al., 2012)
[2] The neural basis of cognitive-emotional processing in people with an at-risk mental state for developing psychosis.

|Table 3.1 Demographic variables, mean scores and standard deviations (SD) of alexithymia and affect scores, alexithymia comparisons between males and females and scanner sites (t-tests), and correlations between alexithymia, affect and demographic variables (n=57)

	Mean (SD)	Statistics Cognitive dimension		Statistics Affective dimension	
Demographics					
Gender (male vs. female)	29 vs. 28	t = .94	p = .35	t = .46	p = .65
Age	34.1 (10.9)	r =-.12	p = .36	r = .17	p = .20
Education (in years)	17.2 (0.7)	r = .03	p = .82	r =-.12	p = .39
Scanner site					
(Groningen vs. Amsterdam)	38 vs. 19	t =-.59	p = .56	t = .74	p = .46
BVAQ					
Total score	91.3 (16.5)	r = .85	p < .01	r = .64	p < .01
Cognitive dimension	49.9 (12.9)		r = .13	p = .34	
Affective dimension	41.2 (8.8)	r = .13	p = .34		
Identifying factor	14.7 (5.0)				
Verbalizing factor	19.8 (6.8)				
Analyzing factor	15.5 (4.3)				
Emotionalizing factor	19.9 (4.0)				
Fantasizing factor	21.3 (7.2)				
PANAS					
Positive affect		r =-.06	p = .65	r =-.04	p = .77
Negative affect		r = .16	p = .22	r = .05	p = .73

Abbreviations: BVAQ: Bermond-Vorst Alexithymia Questionnaire; PANAS: positive and negative affect scale; SD: standard deviation

alexithymia. The BVAQ consists of five subscales (eight items per scale), identifying, verbalizing, analyzing, emotionalizing and fantasizing as defined by Nemiah and Sifneos (Nemiah and Sifneos, 1970). Answers are rated on a 5-point Likert scale (1=certainly does not apply to me, 5=certainly does apply to me) with higher scores indicating more pronounced alexithymic characteristics. Previous studies have confirmed the five-factor structure of the BVAQ and have shown that the BVAQ has good psychometric properties (Berthoz et al., 2000; Vorst and Bermond, 2001).

Using the BVAQ, a second-order distinction can be made in which the factors emotionalizing and fantasizing are grouped into the affective dimension, and the factors identifying, verbalizing, and analyzing feelings into the cognitive dimension of alexithymia. The validity of this two-factor structure has been demonstrated and confirmed by several factor-analyses (Bailey and Henry, 2007; Bekker et al., 2007; Bermond et al., 2007), although not consistently (Bagby et al., 2009). Deborde et al. (2008) developed cut-off scores based on the BVAQ-B. The BVAQ-B is a shorter version of the BVAQ which is calculated by adding the scores on items 21 through 40 (Zech et al., 1999). The complete BVAQ scores were applied in all the analyses in the current study. However, to give an indication of severity of alexithymia in our sample, BVAQ-B scores were calculated. A score of 53 or higher indicates

high alexithymia, while participants scoring 43 or lower can be classified as low alexithymic (Deborde et al., 2008).

Positive and Negative Affect Scale

The positive and negative affect scale (PANAS) (Watson et al., 1988) was used to measure the current affective state. The scale consists of 10 positive affect items (reflecting the extent to which a person feels enthusiastic, active and alert) and 10 negative affect items (reflecting distress, anger, fear and guilt). Participants rated on a five-point scale to what extent they experienced certain mood states. The PANAS has been proven to be a reliable and valid measure of positive and negative affect (Crawford and Henry, 2004).

Image acquisition

Imaging data were acquired using 3.0 Tesla magnetic resonance imaging systems (Philips Intera, Best, NL) located at the University Medical Center Groningen and at the Academic Medical Center in Amsterdam. Both systems were equipped with an 8-SENSE head coil and anatomical images were obtained using a sagittal 3-dimensional T1-weighted sequence (176 slices; TR=9 ms; TE=3.5 ms; FOV=256 mm, voxel size=1 x 1 x 1 mm; slice thickness=1.0 mm).

Statistical analyses

Demographic data were analyzed using SPSS 20 (SPSS Inc, Chicago, Illinois). Pearson correlations were calculated to examine the associations between the two alexithymia dimensions and age, education and PANAS scores. Two-sample t-tests were calculated to examine possible alexithymia differences due to gender or scanner site. Significance was set at $p<.05$, two-sided.

Imaging data were analyzed with unified voxel-based morphometry (VBM) using Statistical Parametric Mapping (SPM8) (http://www.fil.ion.ucl.ac.uk) running under Matlab7 (The MathWorks Inc., Natick, MA, USA). Before processing the data, all images were checked for artifacts and the image origins were manually set at the anterior commissure. Subsequently, images were segmented into gray matter, white matter, and cerebrospinal fluid. The Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) approach was used for optimal registration of individual segments to a group mean template. DARTEL normalized and modulated gray and white matter segments were further normalized to the Montreal Neurological Institute (MNI) space and smoothed using an 8 mm full width half maximum (FWHM) Gaussian kernel. An 8 mm smoothing kernel is optimal for detecting morphometric differences in both large and small neural structures (Honea et al., 2005; White et al., 2001). However, with a smoothing kernel of 8 mm it is possible to miss differences in smaller structures such as the amygdala (Morawetz et al., 2008). Therefore, an additional analysis was performed to specifically investigate the amygdala with a smoothing kernel of 4 mm in order to ensure that differences in this structure were not overlooked.

To assess the effect of alexithymia on gray matter volume (GMV) and white matter volume (WMV), whole brain multiple regression analyses were conducted with the mean-centered cognitive and affective dimension of the BVAQ as covariates of interest. Both the effects of these dimensions separately, as well as the interaction between them were examined. An additional regression model was created including the total BVAQ score to

examine the effect of alexithymia irrespective of subtypes on brain volume. In all abovementioned analyses, sex, age and scanner site were entered as nuisance variables to adjust for their effect on regional brain tissue volumes. Whole brain volume (calculated as the sum of gray and white matter) was entered as a global by means of proportional scaling. A GM majority optimal threshold mask, created based on the whole sample, was applied to all analyses to eliminate voxels of non-GM for the GMV analyses while a white matter majority mask was applied in the WMV analyses (Ridgeway et al., 2009). To examine whether there was an interaction between the affective and cognitive dimension on GMV or WMV, additional analyses were performed by adding the interaction term (mean-centered affective dimension*mean-centered cognitive dimension) to the regression model. Follow-up analyses were performed to examine whether the observed correlations with the cognitive or affective alexithymia dimension were driven by specific BVAQ subscales. Individual volumetric measurements (extracted with masks based on the significant clusters) were entered into SPSS. These measurements were transformed into standardized residuals corrected for gender, age, scanner site, total brain volume, and the respective other alexithymia dimension to mimic the regression values of the SPM-model. For every significant cluster, a multiple regression was performed using a backward elimination procedure with a standard p-to-leave of .1. For significant clusters in association with the cognitive alexithymia dimension, the three subscales 'Identifying', 'Analyzing', and 'Verbalizing' were added into the regression model. For the affective dimension analysis, a regression model including the factor 'Emotionalizing' and 'Fantasizing' was created. To examine main effects of scanner site, a two-sample t-test was performed between the scanner sites in Groningen and Amsterdam in which whole brain volume (GM + WM) was entered as a global by means of proportional scaling.

The threshold for all whole brain analyses was set at $p<.05$ Family-Wise Error (FWE) corrected at the cluster level (corrected for non-stationary of smoothness characteristic for VBM data) with an initial voxel threshold of $p<.001$. For the GMV-analyses, effects occurring in our a priori-set ROIs [bilateral ACC, medial OFC, insulae, and amygdalae as defined by the Automated Anatomical Labeling system implemented in the WFU pickatlas (http://fmri.wfubmc.edu/software/PickAtlas)] had to meet $p<.019$ FWE corrected for the spatial extent of the ROI to be considered significant. This $p=.019$ was chosen to correct for the number of ROIs (i.e. 4) while taking into account their non-independency of the dependent measure [i.e. total GM volume of AAL masks: mean correlation $r=.28$, corrected for total brain volume (http://www.quantitativeskills.com/sisa/index.htm)]. For the WMV-analyses, only one a priori-set ROI was defined (corpus callosum, mask was manually created based on the average template of the whole sample). Effects for this ROI-analysis had to meet $p<.05$ FWE corrected for the spatial extent of the ROI to be considered significant.

To visualize the associations found with the alexithymia dimensions, individual volumetric measurements (extracted with masks based on the significant clusters) were entered into SPSS, corrected for gender, age, scanner site, total brain volume, and the respective other alexithymia dimension, and plotted against the alexithymia dimension of interest.

|RESULTS

Alexithymia scores

The mean scores and standard deviations of the two alexithymia dimensions and the five subscales are presented in Table 3.1. Overall the BVAQ scale demonstrated satisfying internal

|Table 3.2 Internal consistency coefficients (Cronbach's alpha's) of the BVAQ total score, dimensions and subscales

	Cronbach's alpha
BVAQ Total score	.85
BVAQ Cognitive dimension	.89
BVAQ Affective dimension	.78
BVAQ subscales	
Identifying factor	.82
Verbalizing factor	.87
Analyzing factor	.74
Emotionalizing factor	.58
Fantasizing factor	.86

Abbreviations: BVAQ: Bermond-Vorst Alexithymia Questionnaire

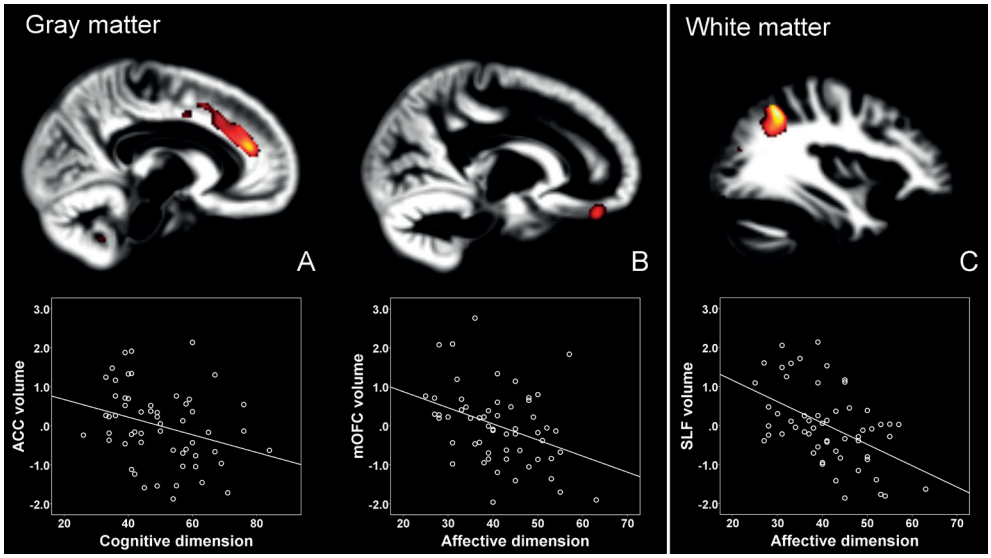
consistencies (see Table 3.2) apart from the subscale 'Emotionalizing' which showed lower internal consistency (α=.58). The two alexithymia dimensions were not significantly related to any of the demographic variables, including sex, age, education and scanner site (see Table 3.1 for test statistics). Furthermore, the two alexithymia dimensions were not significantly correlated with each other nor with the positive and negative affect scores of the PANAS (see Table 3.1). Of our 60 participants, 14 participants could be classified as alexithymic and 26 as non-alexithymic as indicated by the cut-off scores of Deborde et al. (2008) based on the recalculated scores of the BVAQ-B (Zech et al., 1999).

VBM-results

One subject (low alexithymic) was excluded from the analyses because of poor data quality. Furthermore, two subjects (1 high alexithymic and 1 low alexithymic) were excluded because they were identified as outliers by the homogeneity check (VBM8 toolbox version 435, http://dbm.neur.uni-jena.de/vbm). The final sample therefore consisted of 57 participants (for sample characteristics see Table 3.1). No significant correlations were identified between total brain volume and the cognitive dimension (r=-.038) nor the affective dimension (r=-.059).

Gray matter volume results

Whole brain MRI-analyses revealed no significant associations (positive nor negative) between GMV and the cognitive nor affective dimension. No significant associations (in both whole brain as well as ROI analyses) were found with the total BVAQ score and GMV. Voxel-wise analyses however, revealed that the cognitive alexithymia dimension was negatively correlated to GMV in the dorsal ACC [p=.002; k=532; Z=4.09; (x=11 y=35 z=18); Z=3.75; (x=3 y=30 x=27), p<.019 FWE corrected for the extent of the ROI] (see Figure 3.1). The follow-up analysis indicated that this effect was mainly driven by the factor analyzing feelings (see Table 3.3). With the affective dimension, the voxel-wise analysis only showed a marginally significant negative correlation with GMV in the right medial OFC and the affective alexithymia dimension [p=.027 which was above our significance level of .019; k=59; Z=3.54;



|Figure 3.1 Alexithymia-related volume reductions in the (A) right anterior cingulate cortex associated with the cognitive dimension, (B) the right medial orbitofrontal cortex associated with the affective dimension and (C) the right superior longitudinal fasciculus associated with the affective dimension of alexithymia. Results are displayed at p<.001 uncorrected and overlaid on gray and white matter templates based on the segmented T1 images of all participants. The scatterplots are created based on the standardized residuals of the individual volumetric measures (extracted with masks representing the significant clusters) corrected for gender, scanner, total brain volume and the respective other alexithymia dimension.

(x=15 y=39 z=-21)] (see Figure 3.1). This result was not solely driven by a specific factor (see Table 3.3). No significant interaction of the affective and cognitive dimensions was observed for the GMV analyses. Furthermore, in both voxel-wise whole brain and ROI analyses, no significant GMV differences between the two scanner sites were found. The additional analyses with a smoothing kernel of 4 mm did not reveal any significant associations between amygdala volume and the alexithymia dimensions.

White matter volume results

No significant associations (positive nor negative) between WMV and the cognitive alexithymia dimension nor the BVAQ total scores were found in the whole brain analysis nor in the ROI-analysis on the corpus callosum. However, the whole brain analysis did reveal lower WMV in the right superior longitudinal fasciculus (SLF) near the angular gyrus in association with the affective dimension [k=586; Z=4.51; (x=34 y=-55 z=42); Z=3.86; (x=36 y=-58 z=30)] (see Figure 3.1). This result was particularly driven by the factor fantasizing (see Table 3.3). No significant interaction of the affective and cognitive dimension on WMV was observed. Furthermore, no significant effect of the affective dimension on the corpus callosum was found nor any differences between the two scanner sites on WMV in the whole brain analysis and the ROI analyses.

|Table 3.3 Post-hoc backward elimination results of BVAQ subscales contributing to significant gray matter and white matter clusters

Step	Variable	Beta	t-value	p-value
Anterior cingulate gyrus				
1. removal	Identifying	-.01	-.04	.97
2. removal	Verbalizing	-.05	-.34	.74
3. Final model	Analyzing	-.57	-5.17	p<.001
Medial Orbitofrontal cortex				
1. Final model	Fantasizing	-.29	-2.37	.02
	Emotionalizing	-.32	-2.57	.01
Superior longitudinal fasciculus				
1. removal	Emotionalizing	-.16	-1.34	.18
2. Final model	Fantasizing	-.52	-4.50	p<.001

|DISCUSSION

In this study, we investigated the structural correlates underlying the affective and cognitive dimension of alexithymia. In addition to previous MRI studies, which mainly focused on the cognitive alexithymia dimension, we also examined the morphological correlates of the affective dimension. The present results indicate that the two dimensions of alexithymia show distinct anatomical profiles. The cognitive dimension was associated with lower gray matter volume in the dorsal ACC, while the affective dimension was associated with slightly lower gray matter volume in the medial OFC and lower white matter volume in the SLF near the angular gyrus. The total score of the cognitive and affective dimension together was not significantly related to gray or white matter.

The dorsal ACC is an area involved in various cognitive tasks, such as attention and interference (Bush et al., 2000), as well as emotional processing tasks (Etkin et al., 2011). More specifically, the dorsal ACC monitors and evaluates the emotional meaning of stimuli (Etkin, 2010; Etkin et al., 2011) and gets activated when emotion processing tasks require some form of cognitive control (e.g. emotion regulation) (Urry et al., 2009). Lower gray matter volume in this area has been suggested to result in less efficient emotion regulation (Giuliani et al., 2011b) and difficulties in emotion recognition (Baggio et al., 2012). The underlying cellular basis of volumetric reductions remains poorly understood. Reduced volume can indicate a loss of neurons, which will probably impact the direct function of the area. However, it can also reflect a reduction of dendritic arborization (Kanai and Rees, 2011), which will have an impact on the integrity of the circuit in which this area is involved. This latter would support the theory of Lane et al. (2008), that alexithymia results from a loss of fibers between the ACC and affect-generating brain regions (Lane, 2008). Both ACC activation as well as functional connectivity between the ACC and limbic regions has been reported in emotion evaluation and regulation tasks (Banks et al., 2007; Nomura et al., 2003). Together with the finding of lower dorsal ACC volume in association with cognitive alexithymia reported in previous studies (Borsci et al., 2009; Ihme et al., 2013), this suggests that lower volume in this area may underlie the difficulties in evaluating and regulating emotions that individuals with high cognitive alexithymia experience. It was previously suggested that dysfunctioning of the ACC would result in type-I alexithymia because of its

involvement in both emotional experience and emotional cognition (Bermond et al., 2006; Wingbermühle et al., 2012). Indeed, besides the number of functional imaging studies showing altered dorsal ACC activation in cognitive alexithymia (van der Velde et al., 2013), one study, examining both alexithymia dimensions, showed an association between the affective dimension and dorsal ACC activation (Pouga et al., 2010). However, in the current study no GMV reductions in the ACC associated with the affective dimension were found. Furthermore, a recent VBM study revealed that affective alexithymia was associated with larger volume in the cingulate gyrus (Goerlich-Dobre et al., 2013) and previous structural MRI studies have suggested that lower dorsal ACC volume is associated with schizophrenia (Bora et al., 2011; Glahn et al., 2008), which is related to a pattern of alexithymia scores associated with type-II alexithymia (van der Meer et al., 2009). These results indicate that lower ACC volume underlies cognitive alexithymia and might be particularly related to a profile of high cognitive alexithymia combined with normal or even low affective alexithymia scores (subclinical type-II alexithymia). However, further research on dorsal ACC function and volume is necessary to elucidate the role of this area in type-I and type-II alexithymia.

In association with the affective alexithymia dimension, the results revealed a marginal significant reduction in gray matter volume in the medial OFC. This result is in agreement with the theory of Bermond et al. (2006), whom hypothesized that the medial OFC would be associated with the affective dimension since removal of this area led to flatness of affect without changing emotional cognitions (Trigg, 1970). The OFC has strong connections with the amygdala and other limbic areas and this circuit plays a significant role in the experience of emotions (Price and Drevets, 2012). Furthermore, the medial part of the OFC is responsive to highly salient stimuli and activation in this area reflects emotional arousal (Rothkirch et al., 2012) and emotion induction (Rudrauf et al., 2009). Therefore, disruption of this area or the circuit in which it is involved, might contribute to a reduction of emotional experience associated with emotionalizing. Moreover, the medial OFC is involved in reward imagery (Bray et al., 2010) which might explain the relation between the lack of fantasizing and lower medial OFC volume. Taken together, this makes the medial OFC a likely candidate to underlie the problems with emotionalizing and fantasizing associated with the affective dimension.

Besides lower medial OFC volume, the affective alexithymia dimension was associated with lower white matter volume in the right SLF near the angular gyrus, specifically associated with the fantasizing factor. Via connections to the prefrontal and temporal cortex and the hippocampal complex, the angular gyrus integrates information from different modalities and is involved in a wide range of tasks, such as autobiographical memory and social cognition (Seghier, 2013). Activation in the angular gyrus has shown to reflect both imagery and retrieval success (Huijbers et al., 2011). These imagery processes are generated through the connections between the angular gyrus, medial prefrontal cortex and hippocampal formation via the SLF and the inferior longitudinal fasciculus (Andrews Hanna et al., 2010; Makris et al., 2005). Disruption of these connections, because of lower white matter volume, might therefore impair the function of this network and lead to poor imagination and a restricted fantasy life as reported by individuals with high scores on the affective alexithymia dimension. One previous study examining white matter integrity through diffusion tensor imaging (DTI) in association with the cognitive alexithymia dimension (Kubota et al., 2012) also reported reductions in the SLF near the angular gyrus. However, these reductions were reported on the left side of the brain whereas in the current study, white matter volume reductions on the right side were found. One possible explanation for this difference might be that the left angular gyrus and the regions connected to this area through the left SLF are more involved in language processing (Seghier, 2013). Disruption of this left network might therefore be related to the problems in verbalizing

emotions, which is part of the cognitive dimension. In contrast, the right angular gyrus connected with frontal and temporal regions through the right SLF, is part of the salience network (Seghier, 2013), which might be more related to the affective dimension.

Besides structural differences in the ACC and medial OFC, we were expecting to find differences in the corpus callosum, amygdala and insula. Previous studies on alexithymia showed lower white matter volume (Habib et al., 2003) and lower white matter integrity (Kubota et al., 2012) in the corpus callosum in relation to alexithymia. However, both results were only found in patient groups (multiple sclerosis and schizophrenia), while no differences were reported in the healthy control group (Kubota et al., 2012). This, together with our negative finding, may suggests that volumetric differences in the corpus callosum might be specific to comorbid alexithymia in patient populations. Future studies should further examine this difference between patients and healthy controls. The structural alterations in the amygdala and insula also remain equivocal. Two studies reported larger insula volume (Goerlich-Dobre et al., 2013; Zhang et al., 2011), while others reported smaller insula volume in alexithymia (Borsci et al., 2009; Ihme et al., 2013). Furthermore, one study reported lower gray matter volume in the amygdala in association with alexithymia (Ihme et al., 2013). However, the current study and several previous reports (Borsci et al., 2009; Heinzel et al., 2012; Kubota et al., 2011) failed to link insula or amygdala volume to alexithymia. The negative finding regarding amygdala volume in the current study cannot be explained by smoothing, since applying a smaller smoothing kernel (4 mm) did not reveal any alexithymia-related volume changes in this area. Furthermore, it is unlikely that the above mentioned differences in results between studies can be directly explained by large differences in sample size or applied methods. Thus results regarding structural alterations in the insula and amygdala remain inconclusive. However, our results in line with the negative findings of previous studies (Heinzel et al., 2012; Kubota et al., 2011) do not seem to support the idea that alexithymia is related to morphological differences in these areas which are involved in primary salience detection and interoceptive awareness.

The question remains how the volumetric differences related to alexithymia arise. One possibility is that the volumetric abnormalities are heritable and form the neurobiological basis of alexithymia. Previous research has shown that both brain volume (Batouli et al., 2013) as well as alexithymia (Picardi et al., 2011) are in part heritable. However, the volumetric abnormalities might also be caused by the fact that alexithymic individuals process emotion differently (van der Velde et al., 2013) which may result in a different use of emotional brain regions possibly resulting in volumetric changes. Future research studying brain volume related to alexithymia in a family design or in a longitudinal design is necessary to examine this relationship.

Some limitations of this study should be addressed. First, despite the fact that alexithymia is correlated with depression and anxiety (Berthoz et al., 1999), we did not include depression or anxiety measures in the current study. It should be noted, however, that the participants were selected for not having a psychiatric diagnosis. Second, as far as we know, this is the first imaging study trying to relate structural brain correlates to the different subtypes of alexithymia (e.g. type-I and type-II alexithymia). Unfortunately, this association could only be examined through interaction analyses as the sample was not large enough to compare groups of participants classified as type-I alexithymia versus participants classified as type-II alexithymia. Third, in the current study alexithymia is assessed through a self-report measure. This type of measure relies on reflecting one's own emotions which is limited in individuals with alexithymia. Therefore, we encourage future studies to combine self-reports with observer-rated alexithymia measurements, such as the structured interview based on the Beth Israel Hospital Psychosomatic Questionnaire for alexithymia (Sriram et al.,

1988). Finally, the current study used a dimensional approach to examine alexithymia. Although some of the participants exceeded the cut-off for clinical alexithymia [n=13; ≥53 (Deborde et al., 2008)], participants were not specifically selected on this criterion and the sample of 'high' alexithymics was too small to compare directly to non-alexithymics. Therefore, it remains an open question whether the currently identified morphological correlates also apply to clinical alexithymia.

|CONCLUSION

Our results suggest that there might be different anatomical profiles underlying alexithymia and that alexithymia should not be regarded as a unitary construct. The cognitive emotion processing difficulties might be explained by lower gray matter volume in a region involved in emotion recognition and regulation. Whereas affective alexithymia appears to be associated with lower volume in an emotion induction region and lower white matter in a tract connecting regions of a fantasizing network. These results support the idea that the two alexithymia dimensions, that have been identified psychometrically, are associated with specific brain morphology. However, more research is necessary to further elucidate the morphological correlates of the two alexithymia dimensions, especially focusing on the specific relations in highly alexithymic persons. Future research should directly compare groups of individuals with type-I and type-II alexithymia in order to elucidate the neural systems underlying the different alexithymia subtypes and their specific relation to psychopathology.

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4 Alexithymia influences brain activation during emotion perception but not regulation

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|ABSTRACT

BACKGROUND: Alexithymia is a psychological construct which can be divided into a cognitive and affective dimension. The cognitive dimension is characterized by difficulties in identifying, verbalizing and analyzing feelings. The affective dimension comprises reduced levels of emotional experience and imagination. Alexithymia is widely regarded to arise from an impairment of emotion regulation. This is the first functional magnetic resonance imaging (fMRI) study to critically evaluate this by investigating the neural correlates of emotion regulation as a function of alexithymia levels. The aim of the current study was to investigate the neural correlates underlying the two alexithymia dimensions during emotion perception and emotion regulation.

METHODS: Using fMRI, we scanned 51 healthy subjects while viewing, reappraising or suppressing negative emotional pictures.

RESULTS: The results support the idea that cognitive alexithymia, but not affective alexithymia, is associated with lower activation in emotional attention and recognition networks during emotion perception. However, in contrast with several theories, no alexithymia-related differences were found during emotion regulation (reappraisal nor suppression).

CONCLUSION: These findings suggest that alexithymia may result from an early emotion processing deficit rather than compromised frontal circuits subserving higher-order emotion regulation processes.

|INTRODUCTION

Alexithymia ("no words for feelings") is a psychological construct characterized by difficulties in identifying and describing one's feelings, and in distinguishing them from bodily sensations of arousal. Individuals with high scores on alexithymia may further show a lack of imagination and an externally oriented thinking style with a lack of introspection (Sifneos, 1973; Vorst and Bermond, 2001). Alexithymia can be divided into an affective and a cognitive dimension (Vorst and Bermond, 2001). The cognitive alexithymia dimension comprises the subscales difficulties in identifying, analyzing and verbalizing feelings, while the affective dimension consists of the subscales emotionalizing (the degree to which someone is emotionally aroused by emotional stimuli) and fantasizing (the degree to which someone is inclined to imagine). Based on these dimensions, Bermond proposed to distinguish two types of alexithymia [Type-I and type-II; (Bermond et al., 2007)]. Type-I alexithymia is characterized by high scores on both dimensions (i.e. lower cognitive emotional processing capacities and lower emotional arousal). Type-II is characterized by high scores on the cognitive dimension, but normal or even low scores on the affective dimension (i.e. lower cognitive emotional processing capacities, but normal or heightened emotional arousal). Recent studies have suggested that the two alexithymia dimensions might be differently related to the development of psychopathology (Moormann et al., 2008a; van der Meer et al., 2009). Therefore, it is of relevance to gain insight into the neural bases underlying these two dimensions.

Individuals with high scores on alexithymia experience difficulties in emotion processing. Furthermore, alexithymia is generally regarded to be an emotion regulation impairment (Aleman, 2005; Taylor et al., 1997; Taylor and Bagby, 2004; Wingbermühle et al., 2012). However, as far as we know, the neural correlates of emotion regulation in alexithymia through functional neuroimaging, have not yet been investigated.

Emotion processing can be seen as a three-phase process with 1) the identification of the emotional significance of a stimulus, 2) the generation of an affective state, and 3) emotion regulation (Phillips et al., 2003). Previous research has suggested that problems in emotion processing related to alexithymia may already occur during the first two phases of emotion processing. For example, individuals with high scores on alexithymia show deficits in the identification of facial expressions (Grynberg et al., 2012; Swart et al., 2009) and declined attention towards emotional stimuli (Mueller et al., 2006). Furthermore, studies have indicated that individuals with high scores on alexithymia differ in their physiological responses to emotional stimuli (Bermond et al., 2010; Roedema and Simons, 1999), which might reflect differences in the generation of affective states. Phillips and colleagues (2003) suggested that a ventral system, including the amygdala, insula, ventral striatum, ventral anterior cingulate cortex (ACC) and the ventral prefrontal cortex, is involved during the first two phases of emotion processing. Previous studies investigating alexithymia-related brain activation have reported activation differences in this system (Bermond et al., 2006; van der Velde et al., 2013). As far as we know, only one study examined the neural correlates of the two alexithymia dimensions separately through functional magnetic resonance imaging (fMRI) (Pouga et al., 2010), linking the cognitive dimension to lower amygdala activation and the Emotionalizing factor of the affective dimension to higher ACC and lower premotor activation. This indicates that the cognitive and affective alexithymia dimensions may be associated with separable neural correlates during the first two phases of emotion processing.

Emotion regulation, the third phase of emotion processing, is defined as the inhibition or modulation of the affective state and the emotional response (Phillips et al., 2003). Gross

(1998) described reappraisal and suppression, two often applied and widely studied regulation strategies. Reappraisal is a strategy used to change the initial emotional response in such a way that it decreases (e.g. making a negative stimuli less negative), while suppression focuses on inhibiting emotional expressive behavior (e.g. not showing how you feel) (Gross, 1998). Aleman (2005) proposed that alexithymia would be related to difficulties in emotion regulation. Indeed, several studies suggested that individuals with alexithymia seem to apply more suppression, a less efficient strategy, while applying less reappraisal in comparison with non-alexithymic subjects (Kessler et al., 2010; Stasiewicz et al., 2012; Swart et al., 2009; Wingenfeld et al., 2011). However, others have failed to show this association (Geenen et al., 2012; Weiss et al., 2012). During reappraisal, activation in the ventromedial prefrontal cortex and in a dorsal system, including the inferior frontal gyrus, the dorsal ACC, and the dorsal medial frontal cortex, increases (for meta-analysis see Diekhof et al., 2011). By increasing activation in these areas, amygdala activation is consequently reduced, resulting in lower negative affect. Research on suppression indicates increased activation in areas involved in inhibitory control, such as the dorsolateral prefrontal cortex (DLPFC), supplementary motor area, inferior parietal cortex and the precuneus (Goldin et al., 2008; Vanderhasselt et al., 2012). The increased activation in these areas probably results in the restricted facial expression during suppression (Vanderhasselt et al., 2012). Alexithymia-related activation differences during emotion regulation are not yet investigated through fMRI. However, an EEG-study indicated that during reappraisal, P3 amplitudes in the DLPFC, fusiform gyrus, and inferior temporal gyrus decreases in a low alexithymia group, but not in a high alexithymia group (Pollatos and Gramann, 2012). Furthermore, during suppression event-related potentials are negatively correlated to alexithymia (Walker et al., 2011). These findings suggest that alexithymia might be related to differential brain activation patterns during emotion regulation.

The current study is the first fMRI study on emotion regulation in alexithymia. We aimed to investigate neural correlates as a function of the cognitive and affective alexithymia dimension during the different phases of emotion processing. To this extent we presented 51 subjects differing in alexithymia scores with an emotion regulation task. The task consisted of an emotion perception part in which subjects viewed negative or neutral pictures, followed by an emotion regulation part where subjects were instructed to either attend to, reappraise or suppress the emotional content of the picture. We hypothesize that the two alexithymia dimensions are associated with separable neural correlates. We suggest that during the first two phases of emotion processing, the cognitive alexithymia dimension will be associated with decreased activation in brain areas involved in the identification of emotions (e.g. amygdala) while the affective dimension is proposed to be associated with activation in areas involved in emotional awareness (e.g. ACC). During reappraisal we expect to find lower activation in the dorsal system (e.g. DLPFC) associated with the cognitive dimension as reappraisal can be seen as a cognitive task (Ochsner and Gross, 2005).

|METHODS

Participants

Fifty-one healthy subjects were included from a multi-center (Groningen and Amsterdam) add-on study from the GROUP project [Genetic Risk & Outcome of Psychosis, (Korver et al., 2012)]. This sample did not overlap with previous fMRI studies from our group (Goerlich Dobre et al., 2013; Liemburg et al., 2012; Swart et al., 2011). Participants reported no presence or history of any neurological or psychiatric disorder which was confirmed with a

|Table 4.1 Mean scores of demographic data, questionnaire data and rating scores on the emotion regulation task and the associations with alexithymia

Demographic or behavioral variable	Mean (SD)	Statistics Cognitive dimension		Statistics Emotionalizing[a]		Statistics Fantasizing	
Gender (n male)	28	t = 1.28	p = .21	U = 177	p = .006*	t =-.98	p = .33
Handedness (n right)	44	t =-.26	p = .79	U = 133	p = .56	t = .23	p = .82
Scanner site							
(n Groningen)	24	t =-.86	p = .40	U = 312	p = .82	t =-.26	p = .80
Age (years)	37.1 (10.3)	r =-.16	p = .25	ρ =-.01	p = .95	r = .15	p = .30
Education (years)	17.1 (0.8)	r =-.14	p = .34	ρ =-.06	p = .70	r =-.11	p = .42
ERQ							
Reappraisal	5.0 (1.0)	r =-.16	p = .25	ρ = .18	p = .22	r = .07	p = .64
Suppression	2.8 (1.2)	r = .63	p<.001**	ρ = .20	p = .16	r =-.19	p = .18
PANAS							
Positive affect	32.7 (6.0)	r =-.16	p = .26	ρ =-.07	p = .62	r = .16	p = .28
Negative affect	13.1 (4.5)	r =-.09	p = .53	ρ =-.34	p = .02*	r = .26	p = .06
Rating scores							
Attend neutral	1.1 (0.2)	ρ = .21	p = .15	ρ =-.12	p = .41	r =-.06	p = .60
Attend negative	2.6 (0.5)	ρ = -.02	p = .87	ρ =-.28	p = .04*	r =-.04	p = .80
Reappraise	2.1 (0.6)	ρ = .02	p = .90	ρ =-.16	p = .27	r =-.12	p = .62
Suppress	2.4 (0.6)	ρ = .06	p = .65	ρ =-.13	p = .37	r =-.08	p = .81

[a]non-parametric testing due to non-normality; *significant at p<.05, not surviving multiple comparison correction; **significant at p<.001 (controlled for multiple comparisons); *Abbreviations*: ERQ: Emotion regulation questionnaire; PANAS: Positive and negative symptom scale; SD: standard deviation

diagnostic interview. Furthermore, there were no psychotic or mood disorders present in the first-degree relatives of the participants. Demographic variables are presented in Table 4.1.

Behavioral measurements

Diagnostic interviews

During the assessment of the GROUP study (max. two years prior to the fMRI scan) all participants were screened with a diagnostic interview. The SCAN interview [Schedules for the Clinical Assessment of Psychiatry (Wing et al., 1990)] was used to assess the current psychiatric state and psychiatric history of all the participants from the Groningen sample. For the sample from Amsterdam, the CASH [Comprehensive Assessment of Symptoms and History (Andreasen et al., 1992)] was applied for assessing diagnosis and psychopathology. When participants had been given a clinical diagnosis they were excluded from the study. Prior to the fMRI session, participants were asked if there were any changes in their psychological well-being since the last GROUP assessment. If participants reported relevant changes in mood, psychotic symptoms or anxiety, they were excluded from the study.

Bermond-Vorst Alexithymia Questionnaire

The Bermond-Vorst Alexithymia Questionnaire (BVAQ) is a 40-item self-report scale used to assess alexithymia. The BVAQ consists of five subscales (eight items per scale), Identifying, Verbalizing, Analyzing, Emotionalizing and Fantasizing as defined by Nemiah and Sifneos (Nemiah and Sifneos, 1970). Answers were rated on a five-point Likert scale (1=certainly does not apply to me, 5=certainly does apply to me), with higher scores indicating more pronounced alexithymic characteristics. Previous studies have confirmed the five-factor structure of the BVAQ and have shown that the BVAQ has good psychometric properties (Berthoz et al., 2000; Vorst and Bermond, 2001; Zech et al., 1999).

Using the BVAQ, a second-order distinction can be made in which the subscales Emotionalizing and Fantasizing are grouped into an affective dimension, and the subscales Identifying, Verbalizing, and Analyzing feelings into a cognitive dimension of alexithymia. The validity of this two-factor structure has been demonstrated by factor-analyses (Bailey and Henry, 2007; Bekker et al., 2007; Bermond et al., 2007). However, others failed to provide support for this two-factor structure (Bagby et al., 2009; Bekker et al., 2007). In the current sample, the existence of the cognitive dimension was supported by significant correlations between the three subscales of the cognitive dimension ($r=.44$ till $.65$; $p \leq .001$). However, the two subscales of the affective dimension were not correlated to each other ($r=.07$; $p=.62$). Therefore, the Emotionalizing and Fantasizing subscales were treated as separate variables, in line with the approach of previous studies (Bekker et al., 2007; Goerlich-Dobre et al., 2014). In 2008, Deborde and colleagues developed cut-off scores based on the BVAQ-B. The BVAQ-B is a shorter version of the BVAQ, which is calculated by summing up the scores on items 21 through 40 (Zech et al., 1999). To give an indication of severity of alexithymia in our sample, BVAQ-B scores were calculated. A score of 53 or higher indicates high alexithymia, while scores of 43 or lower indicate low alexithymia (Deborde et al., 2008).

Emotion Regulation Questionnaire

The use of the emotion regulation strategies, reappraisal and suppression, was assessed through the Emotion Regulation Questionnaire (ERQ) (Gross and John, 2003). The ERQ consists of 10 items of which four examine suppression and six examine reappraisal. On a seven-point scale subjects had to rate to what extent a certain statement applied to them (strongly disagree till strongly agree). The ERQ has been proven to be a reliable and valid measure of emotion regulation (Gross and John, 2003). To get a clear view on the relationship between the two strategies, the total scores of both subscales were divided by the number of items per subscale.

Positive and Negative Affect Scale

The positive and negative affect scale (PANAS) (Watson et al., 1988) was used to measure the current affective state. The scale consists of 10 positive affect items (reflecting the extent to which a person feels enthusiastic, active and alert) and 10 negative affect items (reflecting distress, anger, fear and guilt). Participants rated on a five-point scale to what extent they experienced certain mood states. The PANAS has been proven to be a reliable and valid measure of positive and negative affect (Crawford and Henry, 2004).

Emotion regulation task

The emotion regulation task (adapted from Ochsner and Gross, 2005) consisted of four

conditions, Attend Neutral, Attend Negative, Reappraise and Suppress. The stimuli were 66 negative (mean valence: 2.54, mean arousal: 5.83) and 22 neutral pictures (mean valence: 5.10, mean arousal: 3.26) from the International Affective Picture System (IAPS). Each trial was constructed as follows. First, a picture appeared with the instruction 'view' (View condition, 2s). After that, the word 'view' changed in one of the following instructions: 'reappraise', 'suppress' or 'attend' (Regulation condition, 4s). Reappraise meant that the participants had to reappraise the picture in such a way that it became less emotionally disturbing. During suppression, subjects were instructed to refrain from expressing their emotions, in a way that bystanders would not be able to read their emotions by looking at their face. During attend, participants just had to look closely at the picture and not change the way they were feeling. The 22 neutral pictures were always paired with the 'attend' instruction. Negative pictures could be paired with either reappraise (22 pictures), suppress (22 pictures) or attend (22 pictures). Following regulation, a black screen appeared (2s). Subsequently, participants were asked to rate how negative they were feeling on a four-point scale (1=not negative at all; 4=extremely negative) (3s). After rating negative affect the word 'relax' appeared (4s) allowing participants to relax, followed by a black screen (0.5s) to alert participants the next trial was coming. A single trial lasted for 15.5 seconds. After 9 or 10 trials, a fixation cross appeared for 20 seconds. The trial order was randomized using a randomized block design.

Prior to the fMRI scan, a short training was given to teach the application of reappraisal and suppression strategies. During this training, participants practiced the different strategies on negative pictures by telling the researchers how they would apply the strategy.

Image acquisition

MRI data were acquired using two 3.0 Tesla whole body scanners (Philips Intera, Best, The Netherlands) located at the University Medical Center Groningen and at the Academic Medical Center in Amsterdam. Both systems were equipped with an 8-SENSE head coil and scan parameters were set identical. The functional images were acquired by a T2-weighted echo producing 37 slices of 3.5 mm thick with no gap. The images were slightly tilted (30 degrees) to prevent artifacts due to nasal cavities. The functional scans were made in the axial plane (TR=2 s; TE=30 s; flip angle (α)=70°; FOV=224.0, 129.5, 224.0; in-plane resolution 64x62 pixels; isotropic voxels of 3.5 mm) and were scanned interleaved. The T1-weighted anatomical image (170 slices; isotropic voxels of 1 mm; TR=9 ms; TE=3.54 ms; α 8°; FOV=256 mm) was acquired in the bicommissural plane, covering the whole brain.

Statistical analyses

Behavioral analyses were performed using SPSS 20 (SPSS Inc., Chicago, IL, USA). To calculate the main effect of condition on the negative affect rating scores from the emotion regulation task, a Friedman's ANOVA was applied due to non-normality of the ratings scores. Post hoc analyses were performed with a Wilcoxon signed-rank test and corrected for multiple testing using a Bonferroni correction. To examine the effect of alexithymia on the rating scores, non-parametric Spearman's correlations were performed. To examine whether the cognitive alexithymia dimension and the Fantasizing subscale were associated with demographic variables, ERQ scores and PANAS scores, two-sample t-tests and Pearson correlations were performed. The associations with the Emotionalizing subscale were calculated using non-parametric tests due to non-normality of this subscale. For all calculated

associations with alexithymia the significance level was set at p<.001 to control for multiple testing (Bonferroni correction for 39 tests). Furthermore, Spearman's correlations were performed between the ERQ reappraisal and suppression scores and the negative affect rating scores of the emotion regulation task. For these analyses the threshold was set to p<.006 (correcting for eight tests).

The fMRI data were analyzed using Statistical Parametric Mapping (SPM 8) (www.fil.ion.ucl.ac.uk) using Matlab 7 (The MathWorks Inc., Natick, MA, USA). Before processing the data, all images were checked for artifacts. Slice timing was applied to the functional images and the functional data were spatially realigned, resliced and coregistered. The anatomical data were segmented. To enhance the accuracy of inter-subject alignment, the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) approach was used to create a gray matter template based on the gray matter segmented images of all subjects. This created template was used to normalize the functional images to and affine-transform them into Montreal Neurological Institute (MNI) stereotactic space. Data were smoothed with a full-width half-maximum Gaussian kernel of 6 mm. Subject head movement greater than 3 mm in more than one direction resulted in exclusion of the data.

Sixteen task-related regressors were modeled with a boxcar function convolving a haemodynamic response function. The regressors View and Relax were divided into View/Relax neutral and View/Relax negative. The other regressors (Regulation, Linger and Rating) were divided into a Reappraise, Suppress, Attend Negative and Attend Neutral part. Additionally, the realignment parameters and the first derivatives thereof were entered as covariates to correct for the effects related to head motion (Friston et al., 1996). Five contrasts were made for each participant: 1) View Neutral versus Baseline; 2) View Negative versus View Neutral; 3) Attend Negative versus Attend Neutral; 4) Reappraise versus Attend Negative; 5) Suppress versus Attend Negative.

To examine task-related activation, one-sample t-tests were conducted. Sex, handedness and scanner site (Amsterdam vs. Groningen) were entered as covariates of no interest. To examine the effect of the cognitive alexithymia dimension on task-related activity (positive and negative correlations), the demeaned scores of the cognitive dimension of the BVAQ were entered as a covariate of interest in a whole brain multiple regression analyses with sex, handedness, scanner site and the sum of the Emotionalizing and Fantasizing subscales as nuisance variables. Additional regression models were created for the three cognitive subscales of the BVAQ separately as covariates of interest to explore whether the observed correlations with the cognitive dimension were driven by specific subscales. To examine brain activation related to the affective alexithymia dimension (positive and negative correlations), two separate multiple regression models were created for the Emotionalizing and Fantasizing subscales. In both models the cognitive dimension, sex, handedness and site were included as nuisance variables.

To limit possible false positives due to multiple comparisons, effects had to meet p<.05 Family-Wise Error (FWE) corrected at the cluster level to be considered statistically significant (initial height-threshold for all the analyses was set at p<.001). Because of specific hypotheses regarding the amygdala, a Small Volume Correction (SVC) was applied if this region would not show in the whole-brain analyses.

|Table 4.2 Mean, standard deviation, range and internal consistency of the alexithymia scores

Variable	Mean (SD)	Range	Cronbach's alpha
Affective dimension	41.6 (8.5)	23- 57	.75
Emotionalizing	20.0 (3.9)	13- 27	.54
Fantasizing	21.6 (7.3)	9- 40	.87
Cognitive dimension	48.0 (13.7)	26- 84	.90
Identifying	13.8 (5.3)	8- 29	.78
Verbalizing	18.9 (6.4)	9- 35	.86
Analyzing	15.3 (5.0)	8- 28	.81
Recalculated BVAQ-B	44.5 (8.6)	29- 62	.73

Abbreviations: BVAQ: Bermond-Vorst alexithymia questionnaire; SD: standard deviation

|RESULTS

Behavioral results

Alexithymia scores

Mean alexithymia scores, range and internal consistencies of the BVAQ are presented in Table 4.2. Alexithymia was not significantly related to any of the demographic variables, including sex, handedness, age and scanner site (see Table 4.1 for test statistics). The Fantasizing and Emotionalizing subscales were not significantly correlated to the cognitive dimension (r=-.13, p=.38; r=.08, p=.61, respectively). Of our 51 participants, 12 participants could be classified as alexithymic and 24 as non-alexithymic as indicated by the cut-off scores of Deborde et al., (2008) based on the recalculated scores of the BVAQ-B (Zech et al., 1999).

The cognitive dimension was positively correlated with the ERQ suppression scores, but not with ERQ reappraisal. The Fantasizing and Emotionalizing subscales did not correlate significantly with either reappraisal or suppression. Furthermore, no significant correlations were found between PANAS positive or negative factors and alexithymia, except from a small negative correlation between the Emotionalizing subscale and negative affect (not surviving multiple comparison correction) (see Table 4.1).

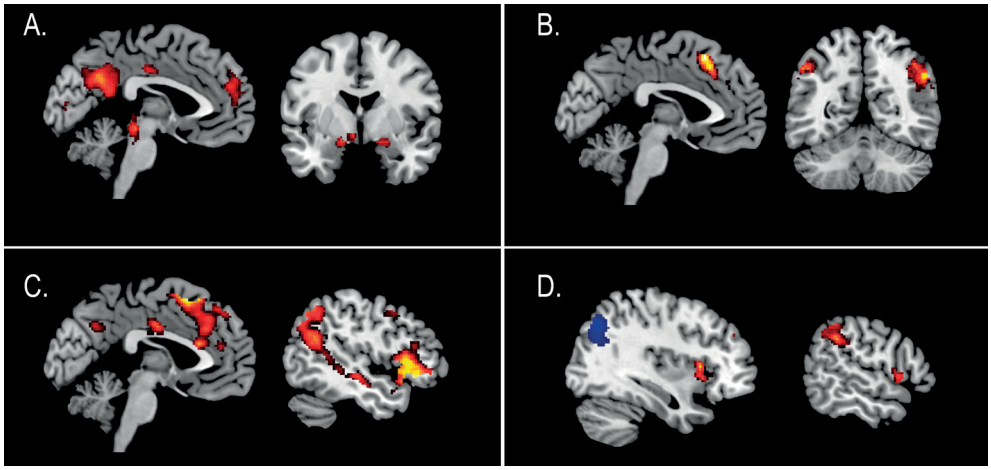
Rating scores of the emotion regulation task

A significant main effect of condition (Attend Neutral, Attend Negative, Reappraise and Suppress) was observed on the ratings of the emotion regulation task [$\chi^2(3)=107$, $p<.001$]. Post hoc tests revealed that negative stimuli were rated more negatively than neutral stimuli ($Z=6.15$, $p<.001$). Furthermore, the participants were able to reduce their negative affect through reappraisal ($Z=-5.24$, $p<.001$) and suppression ($Z=-3.02$, $p=.003$) in comparison with attending negative stimuli. The cognitive dimension, Fantasizing and Emotionalizing were not significantly associated with any of the rating scores, except for a small negative correlation between negative affect scores during attend negative and the Emotionalizing factor (not surviving multiple comparison correction) (see Table 4.1). The correlation between the ERQ reappraisal subscale and the rating scores of negative affect after reappraisal was marginally significant ($\rho=-.356$; $p=.01$, not reaching our threshold of $p<.006$). No other significant correlations were found between the ERQ and rating scores ($p>.1$).

Neuroimaging results

Main effects of emotion regulation task

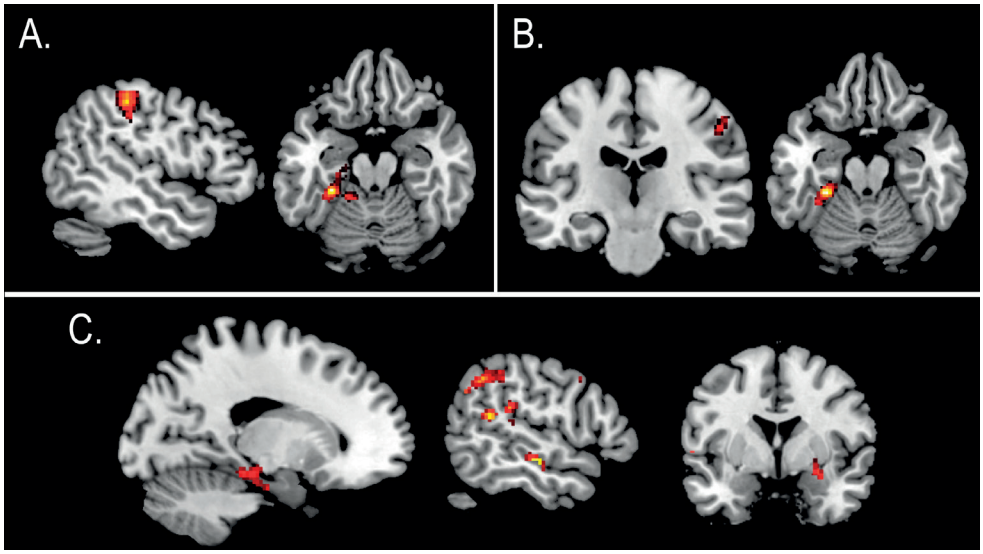
The first two seconds of viewing a negative picture compared with viewing a neutral picture revealed higher activation in various emotion processing areas, such as the right fusiform gyrus, left anterior insula, left middle cingulate gyrus, the bilateral inferior and superior parietal gyrus and the right amygdala (see Figure 4.1A; for more specific details and a full overview of the results, see supplementary Table S4.1). The left amygdala reached significance after applying a SVC (k=29; -16, -2, -14 [x, y, z]; Z=4.33). The four seconds of attending to a negative picture (after two seconds of viewing) yielded significantly higher activation in the left medial frontal gyrus, left middle and inferior frontal gyrus and the bilateral angular and inferior parietal gyrus, in comparison with attending a neutral picture (see Figure 4.1B and supplementary Table S4.1). Reappraising negative pictures in comparison to the attending condition yielded, amongst others, higher activation in the left inferior frontal gyrus, bilateral dorsal medial prefrontal cortex and dorsal ACC (see Figure 4.1C and see supplementary Table S4.1 for full details on all the significant findings). No activation decreases were found for any of the abovementioned contrasts. Suppressing compared with attending negative pictures, led to higher activation in the bilateral insula, supramarginal gyrus, bilateral middle cingulate gyrus, right middle frontal gyrus and the right superior frontal gyrus. Furthermore, activation was lower in the bilateral occipital cortex and the left postcentral gyrus (see Figure 4.1D and supplementary Table S4.1).



|Figure 4.1 Main effects of emotion processing and emotion regulation. (A) Increased activation during negative picture viewing compared with neutral picture viewing. (B) Increased activation during the 3rd till 6th second of attending a negative picture compared with a neutral picture. (C) Increased activation during the reappraisal of a negative picture compared with attending. (D) Increased (red) and decreased (blue) activation during the suppression of a negative picture compared with attending. Results are displayed at p<.001, with a p<.05 FWE correction at cluster level and overlaid on a MNI template brain

Alexithymia-related brain activation

During the contrast 'view negative>view neutral' the cognitive dimension of alexithymia was associated with lower activation in the left inferior parietal cortex, the left fusiform gyrus and the bilateral precuneus (see Figure 4.2A and Table 4.3). This decrease in activation was driven by the subscale Identifying and the subscale Verbalizing. Verbalizing was associated



|Figure 4.2 Alexithymia-related brain activation differences during emotion processing. (A) Decreased activation during negative picture viewing associated with the cognitive alexithymia dimension. (B) Decreased activation during negative picture viewing associated with the Verbalizing subscale. (C) Decreased activation during negative picture viewing associated with the Identifying subscale. Results are overlaid on an MNI template brain and displayed at p<.001, with a p<.05 FWE correction at cluster level, except for the amygdala (right picture under C), which displays the result of the SVC

with lower activation in the right precuneus, left fusiform gyrus and the right postcentral gyrus (see Figure 4.2B and Table 4.3). The Identifying subscale correlated negatively with various emotion processing areas such as, the bilateral inferior frontal gyrus, left parietal cortex, the right precuneus, the right supramarginal gyrus and left superior temporal cortex (see Figure 4.2C and Table 4.3). The amygdala was not found to be significantly correlated to alexithymia in the whole brain analyses. However, significant lower activation in the right amygdala in association with the Identifying subscale was reached after applying a SVC (k=25; 28, 0, -12 [x, y, z]; Z=4.12). No positive correlations were found with the cognitive dimension. Furthermore, the Fantasizing and Emotionalizing subscales did not correlate (positive nor negative) with brain activation during negative emotion picture viewing. Scatterplots of all alexithymia-related brain activation differences are depicted in supplementary Figure S4.1-S4.3. The Identifying subscale contained one outlier (>3 s.d.). To examine the influence of this outlier, the Cook's distance was calculated and the scatterplots were visually inspected. This procedure resulted in the exclusion of two findings (depicted, in gray, in supplementary Figure S4.2). No significant alexithymia-related activation (positive nor negative) was found during the Attend, Reappraise or Suppress condition with either the cognitive dimension nor the Fantasizing and Emotionalizing subscales.

|DISCUSSION

The aim of the current study was to examine neural correlates as a function of alexithymia during emotion perception and regulation. Furthermore, we wanted to investigate whether these neural correlates differed for the cognitive dimension and the subscales of the affective alexithymia dimension. Our results indicate that the cognitive alexithymia dimension is

|Table 4.3 Summary of significant findings of neural correlates associated with alexithymia during negative emotion processing

		MNI coordinates			
Brain region	**k voxels**	**x**	**y**	**z**	**Z**
Negative correlation with cognitive dimension					
L Inferior parietal gyrus	146	-52	-30	46	4.67
R Precuneus	160	8	-72	52	4.67
		10	-66	58	3.98
		8	-62	36	3.74
L Fusiform gyrus	197	-30	-36	-20	4.60
		-16	-20	-12	3.84
		-20	-40	-20	3.83
L Precuneus	144	-12	-46	64	3.94
		4	-50	64	3.66
		-28	-38	66	3.61
Negative correlation with identifying					
L Superior temporal gyrus	94	-56	-6	0	5.30
L Inferior parietal gyrus	338	-52	-28	48	4.72
		-50	-28	38	4.07
		-40	-48	56	3.71
L Middle and inferior frontal gyrus	152	-34	36	18	4.70
		-44	34	22	3.77
		-44	32	30	3.70
R Inferior frontal gyrus and precentral gyrus	140	42	10	34	4.45
		38	16	30	3.46
		56	10	40	3.24
R Supramarginal gyrus and inferior parietal gyrus	231	52	-44	46	4.43
		56	-48	40	3.92
		54	-36	40	3.76
L Parahippocampal gyrus and cerebellum	261	-10	-24	-24	4.27
		-8	-34	-26	4.12
		-22	-20	-22	3.84
R Middle temporal gyrus	97	56	-16	-10	4.20
		46	-10	-10	3.44
		60	-8	-4	3.17

|Table 4.3 Continued

Brain region	k voxels	MNI coordinates x	y	z	Z
R Precuneus	149	2	-66	42	3.86
		4	-60	36	3.85
		10	-66	58	3.73
Negative correlation with verbalizing					
R Precuneus and superior parietal gyrus	162	8	-72	52	4.77
		20	-68	56	3.74
		20	-60	62	3.64
L Fusiform gyrus	135	-28	-36	-20	4.72
		-34	-42	-22	3.97
R Postcentral gyrus	155	52	-20	44	3.77
		50	-28	52	3.64
		40	-26	48	3.49

Abbreviations: L: left; R: right

associated with lower activation in a widespread emotion attention and recognition network during the initial phase of emotion perception. No alexithymia-related brain activation differences were found in a later phase of emotion regulation.

Basic emotion processing

Our emotion perception task reliably activated key regions that have been shown to be involved in emotion processing, such as the amygdala, insula and medial prefrontal cortex (Fusar-Poli et al., 2009; Phan et al., 2002). In association with cognitive alexithymia (Identifying subscale), we found lower activation in a part of the ventral system as defined by Phillips and colleagues (2003), namely the right amygdala, during emotion perception. This is in accordance with previous studies that also reported negative correlations between right amygdala activation and the Identifying subscale (Kugel et al., 2008; Pouga et al., 2010; Reker et al., 2010). The amygdala is an important area in directing bottom-up attention towards emotional stimuli (Vuilleumier, 2005). When presented with a relevant stimulus, the amygdala becomes activated and subsequently activates occipital areas directing visual attention towards the stimulus (Jacobs et al., 2012; Vuilleumier, 2005). Therefore, lower activation in this area might indicate that attention is less automatically directed towards emotional stimuli in alexithymia and might explain the emotional attention deficits in alexithymia as reported by behavioral studies (Mueller et al., 2006; Suslow et al., 2003).

Furthermore, we found lower activation in association with cognitive alexithymia in several other emotion processing areas, such as the inferior parietal lobe, the superior temporal cortex, the somatosensory cortex and the parahippocampal gyrus, which is in accordance with previous findings (Duan et al., 2010; Kano et al., 2003; Reker et al., 2010). According to Adolphs (2002a,b), all of these regions are involved in emotion recognition.

After the fast direction of attention involving the amygdala and occipital cortex, the superior temporal cortex and hippocampal formation become activated to retrieve conceptual knowledge about the emotion and to generate an emotional reaction (Adolphs, 2002b). Subsequently, connections to, amongst others, the motor and somatosensory areas lead to emotional awareness and recognition (Adolphs, 2002a). These results might explain the worse performance of alexithymic individuals on emotional perception and recognition tasks (Lane et al., 1996; Swart et al., 2009) and might account for the problems in recognizing feelings in themselves and others.

Moreover, the dorsolateral prefrontal cortex (DLPFC), part of the dorsal system, was less activated during emotion perception. Together with the lower activation found in the inferior parietal lobe this might suggest less functioning of the frontoparietal attention network (Corbetta, 1998; Corbetta et al., 2008). When attention is not automatically directed towards emotional stimuli, visual attention can be enhanced by this top-down attention network (Ochsner et al., 2009). Lower activation in this network could suggest that individuals with higher scores on alexithymia are overall less inclined to direct their visual attention towards emotional stimuli.

In contrast to our hypotheses, we did not find any significant associations between the Fantasizing or Emotionalizing subscales of the affective dimension and brain activation during emotion perception, while previous studies did suggest specific neural correlates to underlie this dimension (Goerlich et al., 2012; Pouga et al., 2010). These findings are in accordance with the EEG-study of Goerlich and colleagues (2012) in which the cognitive dimension was associated with larger early N1 potentials, while the affective dimension did not influence these early brain potentials. It is possible that the lower activation in the early emotional attention regions is specifically underlying the problems in the cognitive processing of emotion, such as identifying, and that it is less important for the guidance of physiological responses to emotions (e.g. emotion arousal). However, Goerlich and colleagues (2012) showed that the affective dimension was specifically related to a reduction of brain potentials in a later phase of emotion processing (e.g. P3), while we were unable to show activation differences related to either the cognitive or affective (Emotionalizing and Fantasizing) dimension in a later phase of emotion processing (the Attend condition). It could be that these effects were too subtle to detect in the current task. Future research combining fMRI and physiological data could give more insight into these processes. Furthermore, research applying tasks specifically designed to generate affective states or imagination (e.g. Aust et al., 2013; Karlsson et al., 2008; Mantani et al., 2005) might be useful to gain more insight into the neural correlates of the Emotionalizing and Fantasizing subscales. Distinguishing neural correlates related to either the cognitive or affective alexithymia dimension is important to further disentangle alexithymia-related brain activation patterns.

Emotion regulation

To our knowledge, this is the first fMRI study on emotion regulation and alexithymia. During reappraisal, participants activated a large part of the dorsal system, such as the left inferior frontal gyrus and the dorsal ACC, which is in accordance with previous fMRI studies on reappraisal (for meta-analysis see Diekhof et al., 2011). We hypothesized that during reappraisal, activation in this dorsal system would be lower in individuals with high alexithymia scores. However, we were unable to find any alexithymia-related activation differences. This result is in accordance with a recent EEG-study that also did not find any differences in brain potentials during reappraisal associated with alexithymia (Walker et al.,

2011). However, another study reported that low alexithymia was related to reduced P3 and slow wave potentials (Pollatos and Gramann, 2012). These divergent results might be explained by the fact that part of the regions in which decreased brain potentials were found (fusiform gyrus and inferior temporal gyrus) are not generally activated during reappraisal (Diekhof et al., 2011). Furthermore, these regions were not activated in the main effect of the current reappraisal paradigm which might explain the lack of an association between alexithymia and these areas in the current study. The lack of alexithymia-related activation differences during reappraisal, together with the fact that participants with high alexithymia scores were able to down-regulate their negative affect through reappraisal as indicated by the rating scores, suggests that individuals with alexithymia are able to use cognitive reappraisal, at least when they are explicitly trained and cued to do so.

The fMRI results of the main effect of suppression were in accordance with previous literature (Goldin et al., 2008; Vanderhasselt et al., 2012). In relation to alexithymia, no significant activation differences were found. This finding is in contrast with an EEG-study in which lower P2, N2, and late positive potentials were found in association with cognitive alexithymia during suppression (Walker et al., 2011). The discrepancy between the findings of the current study and the study of Walker and colleagues might be explained by the use of a different questionnaire (TAS-20). However, it is unlikely that this difference can fully explain the different findings as the TAS-20 total score and BVAQ cognitive dimension are highly correlated (Vorst and Bermond, 2001). In line with the current fMRI results, no differences in rating scores after suppression were found. This indicates that individuals with higher alexithymia scores were capable of decreasing negative affect through suppression which was also found in the study of Walker et al. (2011). Therefore, these results indicate that individuals with alexithymia do not seem to be impaired in the use of suppression. Future research is necessary to see if this finding can be replicated.

Limitations and future implications

Some limitations of the current study should be addressed. First, we did not include depression or anxiety measures, despite the fact that correlations between these and alexithymia have been found (Berthoz et al., 1999). We should note, however, that none of our subjects had a clinical psychiatric diagnosis of mood disorder or otherwise. Moreover, alexithymia was not related to positive or negative affect prior to scanning, as measured with the PANAS. Second, our results indicate neural correlates to differ already early in time during emotion processing in alexithymia. However, due to the low temporal resolution of fMRI it is not possible to indicate that these differences already occurred very early. Therefore, we suggest that future studies combine EEG and fMRI to gain both good temporal and spatial resolution. Third, the regulation blocks in the current task were too short to calculate reliable connectivity patterns. We suggest that future research should apply tasks with longer regulation periods to examine functional connectivity patterns associated with alexithymia during emotion regulation. Furthermore, the rating scale of negative affect during the emotion regulation task might not have been sensitive enough to detect associations with alexithymia. Use of a larger scale may overcome this. Finally, the neural correlates were examined through a dimensional approach. Although some of the participants exceeded the cut-off score for clinical alexithymia [N=12; ≥53 (Deborde et al., 2008)], participants were not specifically selected on this criterion and no high versus low alexithymia comparison could be made (due to low power). Therefore, future research is necessary to examine whether the intact functioning of emotion regulation areas also applies to clinical alexithymia.

|CONCLUSION

The results of this study showed that the cognitive dimension, but not the subscales of the affective dimension of alexithymia, seems to affect emotion processing during the perception but not the regulation phase. Activation was lower in a widespread emotional attention and emotional recognition brain network, probably underlying emotional attention and identification deficits in alexithymia. Activation of neural networks during the later phase of emotion regulation did not differ, which is at odds with views of alexithymia as an emotion regulation deficit. In sum, these results suggest that alexithymia may result from an early emotion processing deficit instead of deviant activation in networks subserving emotion regulation. Further research is necessary to investigate whether individuals with high alexithymia scores are also capable of regulating their emotions in daily life.

|ACKNOWLEDGEMENTS

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|SUPPLEMENTARY MATERIAL

|Table S4.1 Main effects of negative emotion processing, reappraisal or suppression on BOLD responses

		MNI coordinates			
	k voxels	x	y	z	Z
View Negative > View Neutral					
R Fusiform gyrus, middle and inferior temporal gyrus	1458	54	-60	6	6.64
		46	-70	2	5.64
		42	-46	-16	5.27
L Middle temporal gyrus, middle occipital gyrus	1786	-48	-70	10	6.40
		-48	-60	4	6.33
		-38	-72	6	6.12
L Precuneus	1267	-6	-52	26	5.70
		-4	-48	18	5.25
		-6	-56	36	5.12
L Supramarginal gyrus	313	-62	-26	34	5.55
		-62	-28	26	5.38
L Inferior frontal gyrus	316	-48	10	16	5.46
		-56	20	10	3.42
Brainstem	454	-4	-30	-12	5.43
		6	-30	-10	4.84
		-26	-28	-12	4.45
R Calcarine sulcus, lingual gyrus	551	12	-82	8	5.37
		14	-74	-4	5.23
		20	-64	-8	4.17
Superior medial frontal gyrus	714	-6	50	24	5.84
		8	56	28	4.82
		-6	50	38	4.16
L Calcarine sulcus, occipital cortex	286	-8	-84	6	5.03
		-16	-68	10	3.63
L Inferior frontal gyrus, hippocampus, anterior insula	875	-50	36	6	4.85
		-16	-4	-14	4.62
		-32	18	-14	4.54
R Occipital cortex	108	24	-80	38	4.53
		28	-72	32	3.91
R Supramarginal gyrus	192	62	-30	28	4.53
		64	-20	36	4.36

|Table S4.1 Continued

		MNI coordinates			
	k voxels	**x**	**y**	**z**	**Z**
		64	-28	38	3.66
R Amygdala, hippocampus	100	18	2	-12	4.42
		20	-8	-14	4.07
L Inferior and superior parietal gyrus	101	-32	-48	56	4.38
R Inferior frontal gyrus	96	48	36	0	4.38
R Caudate	103	10	12	0	4.23
L Middle cingulate gyrus	99	0	-16	38	4.16
		-6	-26	42	3.51
Attend negative > Attend neutral					
L Supplementary motor area, superior medial frontal gyrus	414	-2	20	52	4.70
		-2	24	44	4.43
		-8	12	52	3.91
L Middle and inferior frontal gyrus	169	-48	16	42	4.54
		-42	26	40	3.63
		-52	16	34	3.56
R Angular gyrus, inferior parietal gyrus	553	52	-60	36	4.53
		40	-62	46	4.39
		52	-54	44	4.20
L Angular gyrus, inferior parietal gyrus	560	-48	-60	44	4.17
		-46	-52	42	4.03
		-36	-32	42	3.88
Reappraise > Attend negative					
L Inferior frontal gyrus	4157	-46	26	-8	5.84
		-48	22	2	5.63
		-40	-56	24	5.61
Supplementary motor area, dorsal medial prefrontal cortex, dorsal anterior cingulate cortex	2561	-2	14	56	5.76
		14	18	60	5.60
		6	16	60	5.49
L Middle cingulate gyrus	121	-2	-10	34	4.92
		0	-18	28	3.29

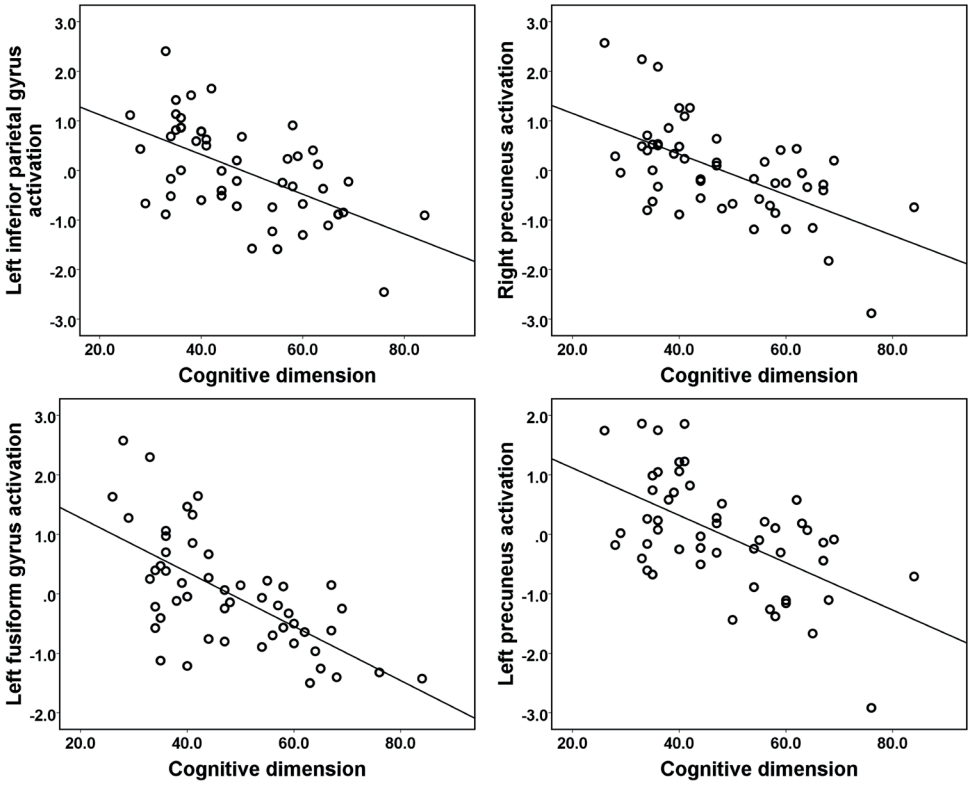
|Table S4.1 Continued

		MNI coordinates			
	k voxels	**x**	**y**	**z**	**Z**
R Caudate	227	14	16	8	4.66
		14	18	0	4.27
		6	2	2	3.34
R Angular gyrus, inferior parietal cortex	382	50	-54	28	4.52
		58	-54	32	4.38
		56	-50	44	3.95
R Middle temporal gyrus	154	44	-34	-4	4.48
		58	-32	-2	3.78
R Orbitofrontal cortex	203	48	42	-6	4.26
		40	22	-12	4.26
		54	34	-4	4.17
L Precuneus	129	-2	-62	34	4.00
		-12	-52	34	3.61
Suppress > Attend negative					
R Anterior insula, rolandic operculum	609	54	10	0	5.10
		46	20	-4	5.03
		38	16	8	4.74
L Anterior insula, inferior frontal gyrus	430	-44	12	0	5.08
		-50	10	6	4.43
		-42	12	-10	4.21
R Supramarginal gyrus	276	60	-40	34	4.85
		58	-42	46	3.98
		60	-32	32	3.81
R Middle frontal gyrus	171	30	54	26	4.64
		32	48	32	4.27
		26	48	22	4.22
Middle cingulate gyrus	327	-4	-16	32	4.49
		4	-24	38	4.40
		-6	-24	24	3.98
Middle cingulate gyrus	569	16	20	58	4.28
		-4	20	36	4.08
		-6	6	60	4.06

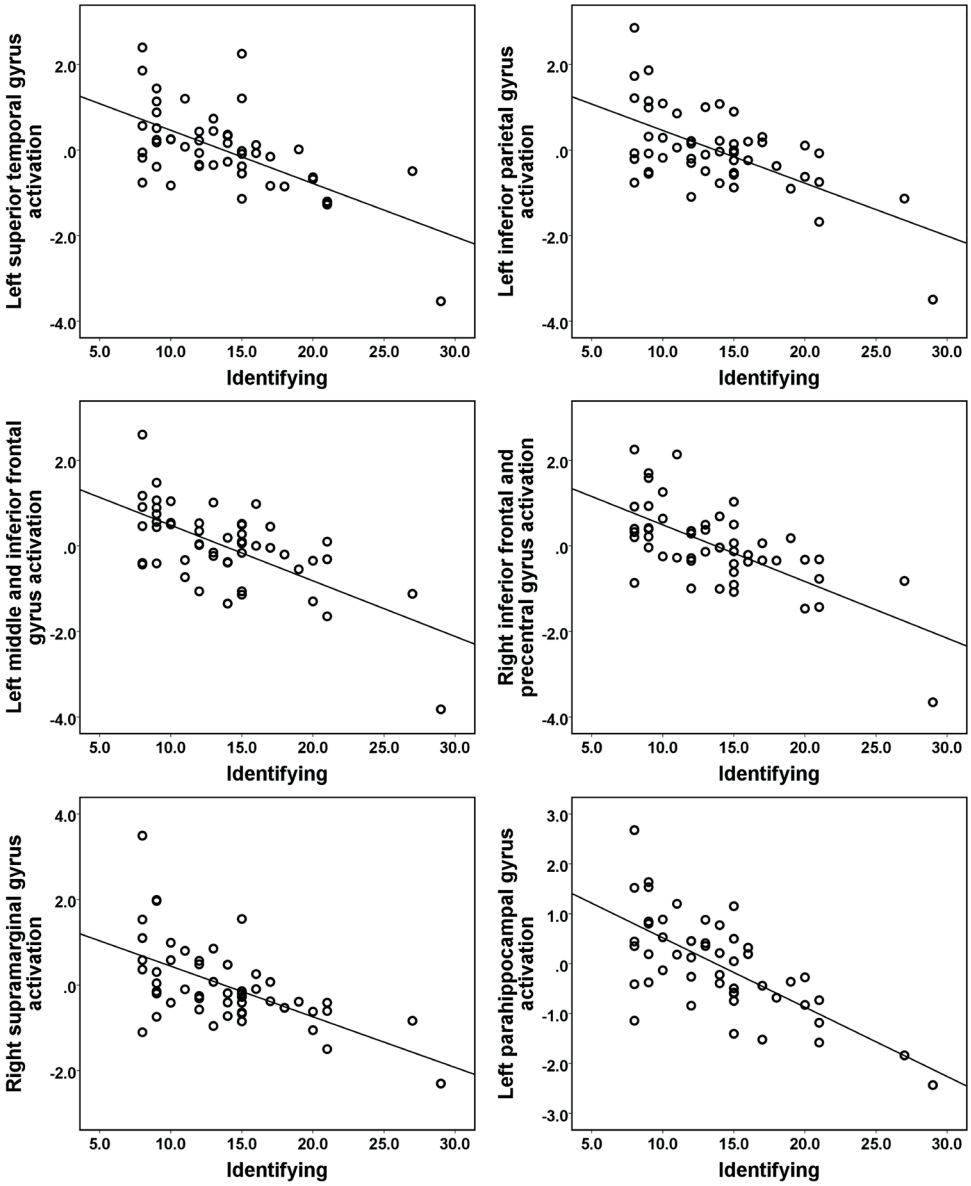
|Table S4.1 Continued

		MNI coordinates			
	k voxels	**x**	**y**	**z**	**Z**
L Supramarginal gyrus	103	-62	40	38	3.82
Attend negative > Suppress					
R Middle occipital gyrus	713	32	-64	36	5.72
		32	-70	30	4.94
		46	-64	30	4.02
L Middle occipital gyrus	306	-30	-60	24	4.19
		-30	-76	32	4.14
		-40	-74	30	4.10
L Postcentral gyrus	295	-44	-28	54	4.13
		-40	-28	46	3.84
		-38	-38	54	3.64

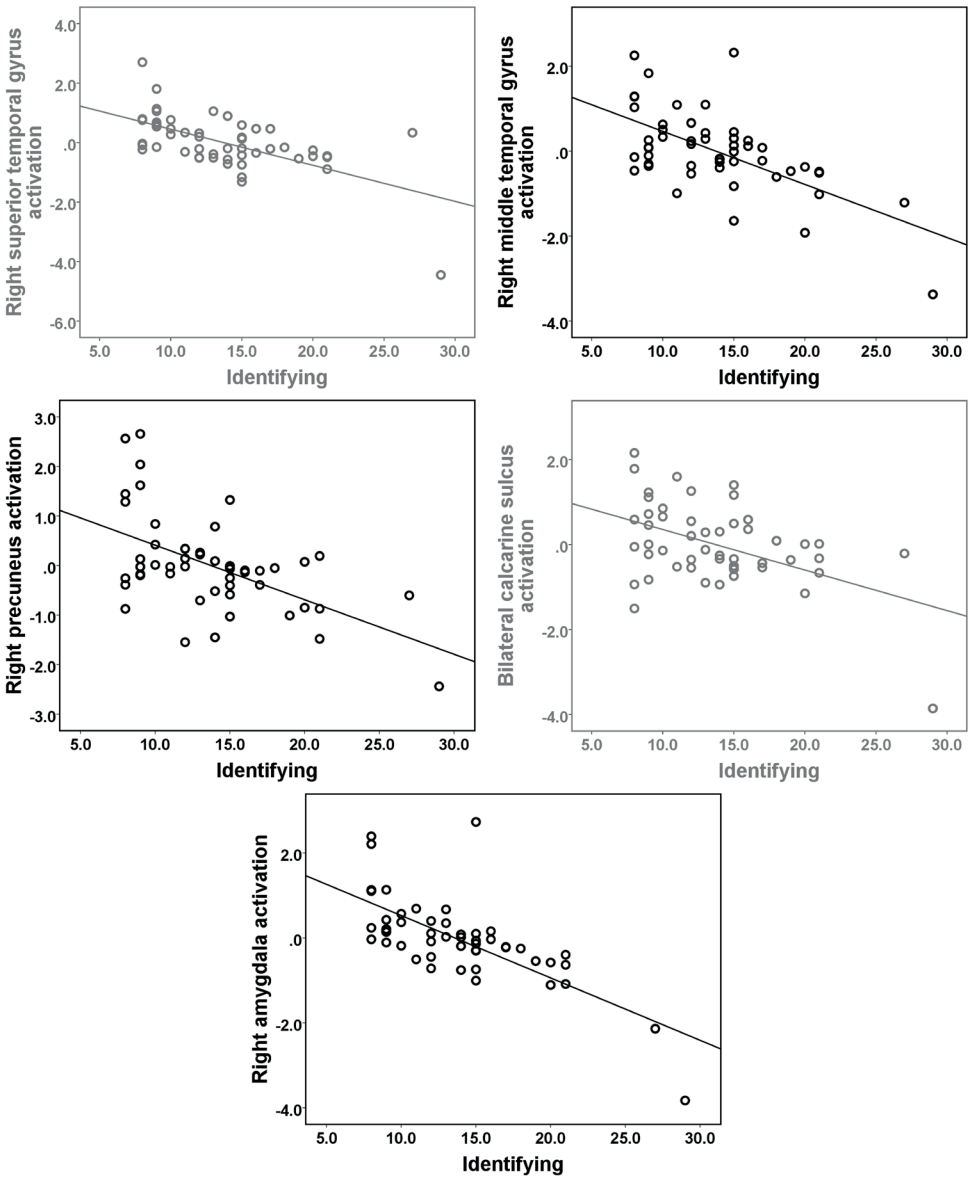
Abbreviations: L: left; R: right



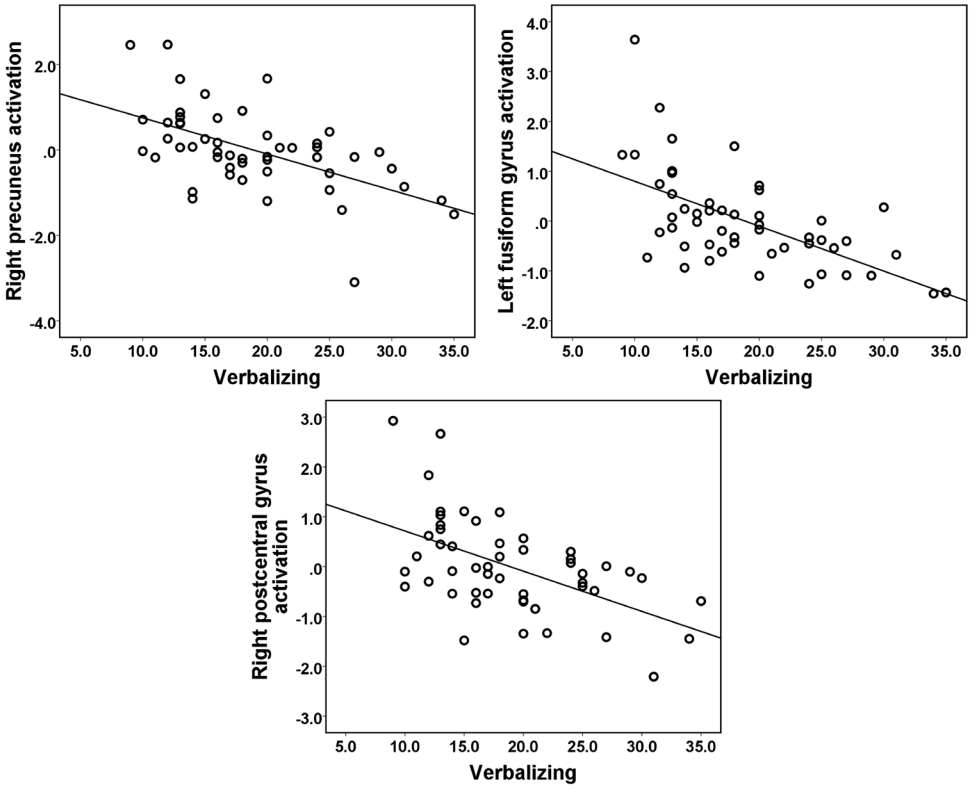
|Figure S4.1 Scatterplots of the cognitive alexithymia scores against the standardized residuals of the individual avaraged cluster activation for the contrast view negative versus view neutral (extracted with masks representing the significant clusters) corrected for gender, scanner, handedness, and the affective alexithymia dimension.



|Figure S4.2a Scatterplots of the identifying alexithymia subscales against the standardized residuals of the individual avaraged cluster activation for the contrast view negative versus view neutral (extracted with masks representing the significant clusters) corrected for gender, scanner, handedness and the affective alexithymia dimension.



|Figure S4.2b For description see supplementary Figure S4.2a. The gray subplots represent excluded findings. These findings were excluded because they were driven by one single outlier (as identified by visual inspection and a Cook's distance >1).



|**Figure S4.3** Scatterplots of the verbalizing alexithymia subscale scores against the standardized residuals of the individual averaged cluster activation for the contrast view negative versus view neutral (extracted with masks representing the significant clusters) corrected for gender, scanner, handedness, and the affective alexithymia dimension.

5 Cognitive alexithymia is associated with the degree of risk for psychosis

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|ABSTRACT

BACKGROUND: Alexithymia is a personality construct denoting emotion processing problems. It has been suggested to encompass two dimensions: a cognitive and affective dimension. The cognitive dimension is characterized by difficulties in identifying, verbalizing and analyzing emotions, while the affective dimension reflects the level of emotional arousal and imagination. Alexithymia has been previously proposed as a risk factor for developing psychosis. More specifically, the two alexithymia dimensions might be differentially related to the vulnerability for psychosis.

METHODS: Therefore, we examined the two dimensions of alexithymia, measured with the BVAQ in 94 siblings of patients with schizophrenia, 52 subjects at ultra-high risk (UHR) for developing psychosis, 38 patients with schizophrenia and 109 healthy controls.

RESULTS: The results revealed that siblings and patients had higher levels of cognitive alexithymia compared to controls. In addition, subjects at UHR for psychosis had even higher levels of cognitive alexithymia compared to the siblings. The levels of affective alexithymia were equal to the controls in siblings and patients. However, UHR individuals had significantly lower levels of affective alexithymia (i.e. higher levels of emotional arousal and fantasizing) compared to controls. Furthermore, alexithymia was related to subclinical levels of negative and depressive symptoms.

CONCLUSION: These findings indicate that alexithymia varies parametrically with the degree of risk for psychosis. More specifically, a type-II alexithymia pattern, with high levels of cognitive alexithymia and normal or low levels of affective alexithymia, might be a vulnerability factor for psychosis.

|INTRODUCTION

Alexithymia is a personality construct characterized by difficulties in verbalizing, identifying and analyzing feelings, as well as a restricted fantasy life (fantasizing) and lower emotional arousal (emotionalizing) (Sifneos, 1973; Vorst and Bermond, 2001). Alexithymia is considered to be a risk factor for several psychiatric and neurological disorders such as depression, anxiety, psychosis and somatic disorders (Taylor et al., 1997). Furthermore, alexithymia is associated with poor social functioning (van Rijn et al., 2011) and lower life satisfaction (Mattila et al., 2007).

It has been suggested that alexithymia is not a unitary construct, but that it can be divided into a cognitive and affective dimension (Vorst and Bermond, 2001). The cognitive dimension refers to the ability to verbalize, identify and analyze feelings, while the affective dimension refers to the level of subjective emotional arousal and the level of fantasizing and daydreaming. Based on these dimensions, different types of alexithymia can be defined (Bermond et al., 2007). Type-I alexithymia is characterized by high scores on both the cognitive and affective dimension indicating difficulties with the cognitive processing of emotions combined with low levels of emotional arousal and daydreaming. Type-II alexithymia, on the other hand, is characterized by high scores on the cognitive dimension, while scores on the affective dimension are normal or low (i.e. high levels of emotional arousal).

Heightened levels of alexithymia have been reported in schizophrenia (Cedro et al., 2001; Kubota et al., 2011; Kubota et al., 2012; van 't Wout et al., 2007; van der Meer et al., 2009; van Rijn et al., 2011; Yu et al., 2011). More specifically, patients with schizophrenia may show a type-II alexithymia profile, indicating difficulties with identifying, analyzing and verbalizing emotions, while levels of emotional arousal and fantasizing are normal (van der Meer et al., 2009) or even heightened (van 't Wout et al., 2007). High levels of subjective emotional arousal in the face of a lack of cognitive emotion processing, may have various negative consequences, such as higher levels of negative affect and anxiety (Montebarocci et al., 2006; Moormann et al., 2008a). It has been suggested that alexithymia might be related to psychotic symptoms in patients with schizophrenia (Picardi et al., 2012; van 't Wout et al., 2007), however not all studies confirm this association (Fogley et al., 2014; Kubota et al., 2011).

Previous research suggested that higher levels of cognitive alexithymia might contribute to a greater vulnerability for psychosis (van 't Wout et al., 2007). Indeed, some studies have shown subjects at increased risk for developing psychosis to have higher scores on cognitive alexithymia. For example, male siblings of patients with schizophrenia, with an increased genetic risk for developing schizophrenia, showed higher levels of difficulties with verbalizing emotions compared to controls (van 't Wout et al., 2007). Furthermore, subjects with an ultra-high risk (UHR) for developing psychosis, appear to have more difficulties in both verbalizing as well as identifying emotions (van Rijn et al., 2011).

Siblings of patients with schizophrenia are at increased risk for developing psychosis with one-year transition rates to psychosis between 0.34 and 4.9 percent (Johnstone et al., 2005; van Nierop et al., 2013). Transition rates to psychosis in the UHR group are even higher with 7 to 40 percent after 1 year (Yung et al., 2005; Yung et al., 2007). If alexithymia indeed contributes to a greater vulnerability for psychosis, one would expect higher alexithymia scores in subjects at UHR for psychosis, compared to siblings. However, to the best of our knowledge, no studies have yet directly compared alexithymia scores between these groups.

The aim of the current study was to examine alexithymia in subjects at genetic risk for

psychosis (siblings), subjects at UHR for psychosis and patients with schizophrenia. We hypothesized that the two high-risk groups and the patient group would show a type-II alexithymia pattern. Furthermore, we hypothesized to find a parametric effect of risk on alexithymia scores, with siblings scoring higher compared to controls, UHR individuals scoring higher compared to siblings and patients scoring higher compared to all three groups (controls < siblings < UHR < patients). Finally, we examined whether alexithymia is related to subclinical and clinical psychotic symptoms.

|METHODS

Participants

In the current study we included 109 healthy controls, 94 siblings of patients with schizophrenia, 52 individuals at UHR for developing psychosis and 38 patients with a DSM-IV diagnosis of schizophrenia. The present data were taken from separate studies from our group (van der Meer et al., 2013; van der Velde et al., 2014; van Rijn et al., 2011). There was a partial overlap of data from the UHR group with the results presented in van Rijn et al., 2011 (n=34 UHR individuals). The patient, sibling and control samples were independent from earlier alexithymia studies published by our group (van 't Wout et al., 2007; van der Meer et al., 2009).

All 94 siblings and 57 controls participated in a multi-center (Amsterdam and Groningen) add-on study of the Genetic Risk and Outcome of Psychosis (GROUP) project (Korver et al., 2012). The other 52 control subjects were recruited via advertisements. Healthy controls and siblings were excluded if they reported a presence or history of psychiatric or neurological disorders. Sixteen UHR subjects were recruited from the Mental Health Care services in Friesland. Subjects with a 6 or higher on the Prodromal questionnaire (PQ-16, Ising et al., 2012) were assessed with the Comprehensive Assessment of At Risk Mental State (CAARMS, Yung et al., 2005) to determine if they met the UHR criteria. The selection procedure was in accordance with the EDIE-NL trial (see Rietdijk et al., 2010 for further details). The inclusion procedure of the other 36 UHR subjects was based on the Structured Interview for prodromal symptoms and the Bonn Scale for the Assessment of Basic Symptom-Prediction List and is described in van Rijn et al. (2011). All the patients with schizophrenia were included from psychiatric institutions across the Netherlands. The clinical diagnosis of the patients was confirmed with the Mini-International Neuropsychiatric Interview (MINI-Plus; Sheehan et al., 1998).

All participants gave written informed consent and all studies were approved by either the local medical ethical committee or the Mental Healthcare Research Ethics Committee (METIGG). All procedures were carried out according to the declaration of Helsinki. Demographic characteristics of the subjects are presented in Table 5.1.

Behavioral measurements

Bermond-Vorst Alexithymia Questionnaire

The Bermond-Vorst Alexithymia Questionnaire (BVAQ) is a 40-item self-report scale used to assess alexithymia. The BVAQ consists of five subscales (eight items per scale), identifying, verbalizing, analyzing, emotionalizing and fantasizing as defined by Nemiah and Sifneos (Nemiah and Sifneos, 1970). Participants rated on a 5-point Likert scale to what extent the statements applied to them (1=certainly does not apply to me, 5=certainly does apply to

me). Higher scores on the BVAQ indicate more pronounced alexithymic characteristics. Previous studies have confirmed the five-factor structure of the BVAQ and have shown that the BVAQ has good psychometric properties (Berthoz et al., 2000; Vorst and Bermond, 2001).

Using the BVAQ, a second-order distinction can be made in which the factors emotionalizing and fantasizing are grouped into the affective dimension, and the subscales identifying, verbalizing, and analyzing feelings into the cognitive dimension of alexithymia. The validity of this two-factor structure has been demonstrated and confirmed by several factor-analyses (Bailey and Henry, 2007; Bermond et al., 2007), however not all studies have replicated this (Bagby et al., 2009).

Community Assessment of Psychic Experiences

The community assessment of psychic experiences (CAPE) is a 42-item self-report questionnaire, which was applied to examine self-reported psychotic-like experiences in the controls and siblings (Stefanis et al., 2002). The frequency of positive, negative and depressive symptoms was measured on a 4-point scale (1=never; 4=nearly always). The average score per subscale (the frequency of positive, negative and depressive symptoms) was used in these analyses.

Positive and negative syndrome scale

To examine the clinical characteristics of the UHR individuals and patients with schizophrenia, the semi-structured interview Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987) was administered. In this 30-item interview, positive, negative and general symptoms of psychosis that occurred in the past week were measured.

Statistical analyses

To examine possible differences between the groups on demographic variables, two analyses of variance (ANOVA) were performed with age and education as dependent variables and group (controls, siblings, UHR, patients) as an independent variable. Furthermore, a chi-square test was performed to examine gender differences between groups. For both analyses the statistical significance level was set at $p<.05$. The results revealed significant group differences on demographic variables (see Table 5.1). Therefore, these were included in further analyses.

To examine group differences on the alexithymia dimensions, two ANCOVA's were performed. The first analysis contained the cognitive alexithymia dimension as a dependent variable and group and sex as independent variables. The second analysis, contained the affective alexithymia dimension as a dependent variable. Age and education were included as covariates in both ANCOVA's. If the ANCOVA's resulted in a significant main effect ($p<.05$), post-hoc comparisons, comparing the groups to each other, were performed. The significance level for these post-hoc tests was set at $p<.05$, corrected for multiple comparisons applying a Bonferroni correction.

To examine whether possible differences were driven by specific subscales, two MANCOVA's were performed. In the first analysis, the cognitive BVAQ subscales (i.e. identifying, verbalizing, analyzing) were included as dependent variables and gender and group were included as independent variables (3x4x2 design). In the second MANCOVA, the

affective BVAQ subscales (i.e. emotionalizing and fantasizing) were included as dependent variables resulting in a 2x4x2 design. In both MANCOVA's, age and level of education were included as covariates. The main effects of gender and group on alexithymia, as well as the interaction between the two were examined at a significance level of $p<.05$. Only if the MANCOVA's resulted in a significant multivariate effect, univariate group effects were examined. For all univariate tests which showed a significant effect of group on alexithymia scores ($p<.05$), post-hoc comparisons were performed, comparing the groups to each other. The significance levels of the post-hoc tests were corrected for multiple testing using a Bonferroni correction. All abovementioned analyses were also repeated without including sex, education and age to examine the effects of the included covariates on the findings.

To examine the correlation between alexithymia and CAPE or PANSS scores, Spearman's rho correlations were performed. Non-parametric testing was chosen because the CAPE scores and the PANSS scores were not normally distributed. For the controls and siblings, correlations between the two alexithymia dimensions and positive, negative and depressive CAPE scores were examined. The significance level was set at $p<.008$ to correct for multiple comparisons (Bonferroni correction on 6 tests). In the UHR individuals and schizophrenia patients, the correlations between the two alexithymia dimensions and the positive, negative and general PANSS scores were examined. The significance level was set at $p<.008$ to correct for multiple comparisons (Bonferroni correction on 6 tests).

|RESULTS

Demographic data

The results revealed a significant main effect of group on age and education (see Table 5.1). Furthermore, the groups differed significantly on gender (see Table 5.1). Therefore, age, education and gender were included in all further analyses.

The cognitive and affective alexithymia dimension

The alexithymia data were checked for outliers (>3 s.d.), no outliers were found on the cognitive dimension, the affective dimension or the alexithymia subscales in any of the groups. Correlation analyses revealed significant associations between the three cognitive subscales (verbalizing – identifying: $r=.56$, $p<.001$; verbalizing – analyzing: $r=.53$, $p<.001$; identifying – analyzing: $r=.44$, $p<.001$) and the two affective subscales (fantasizing – emotionalizing: $r=.24$, $p<.001$), confirming the two-factor structure proposed by Vorst and Bermond (2001). Furthermore, the cognitive and affective alexithymia dimensions were only weakly correlated to each other ($r=.13$, $p=.02$).

The results showed a significant main effect of group on the cognitive alexithymia dimension (see Table 5.1). Furthermore, a significant main effect of gender ($F_{1,283}=15.1$, $p<.001$) on the cognitive dimension was found (i.e. men had higher scores than women), while the main effect of age ($F_{1,283}=.30$, $p=.59$), education ($F_{1,283}=2.5$, $p=.12$) and the group*gender interaction ($F_{3,283}=1.3$, $p=.26$) were not significant. Post-hoc comparisons revealed that all groups (siblings, UHR and patients) had significantly higher cognitive alexithymia scores compared to controls (see Table 5.2). Furthermore, the UHR group had higher cognitive alexithymia scores than siblings, while the patients with schizophrenia did not significantly differ from the siblings or the UHR group (see Table 5.2). Repeating the analyses without including gender, age and education revealed the same significant main

|Table 5.1 Means and standard deviations of demographic variables and alexithymia scores per group and test statistics of group differences.

	HC (n=109)	Siblings (n=94)	UHR group (n=52)	Patients (n=38)	Test statistic
Demographics					
Gender (% male)	50	46	56	76	χ^2=12.9; p=.005
Age	31.4 ± 10.4	32.1 ± 8.0	17.8 ± 4.5	34.4 ± 10.6	$F_{3,289}$=38.0; p<.001
Education[a]	6.0 ± .8	5.9 ± .8	5.2 ± .9	5.3 ± 1.1	$F_{3,289}$=14.6; p<.001
Alexithymia					
Cognitive dimension	48.8 ± 13.0	55.2 ± 15.8	68.0 ± 16.3	64.6 ± 16.4	$F_{3,283}$=14.8; p<.001
Verbalizing	19.7 ± 6.9	21.5 ± 7.2	26.7 ± 7.8	23.8 ± 7.2	$F_{3,283}$=6.9; p<.001
Identifying	14.6 ± 4.6	15.5 ± 5.4	22.1 ± 6.7	21.1 ± 5.9	$F_{3,283}$=17.4; p<.001
Analyzing	15.9 ± 5.3	17.6 ± 5.7	19.3 ± 6.4	19.7 ± 6.8	$F_{3,283}$=3.0; p=.03
Affective dimension	44.5 ± 11.0	43.4 ± 9.9	39.6 ± 10.0	42.5 ± 10.3	$F_{3,283}$=5.3; p=.001
Fantasizing	22.4 ± 7.2	23.0 ± 7.4	18.8 ± 6.7	21.7 ± 6.6	$F_{3,283}$=2.7; p=.047
Emotionalizing	20.7 ± 4.7	20.4 ± 4.9	19.3 ± 6.4	20.8 ± 6.4	$F_{3,283}$=.93; p=.43
CAPE scores (HC: n=66; Siblings: n=84)					
Positive symptoms	1.1 ± .16	1.1 ± .13	N.A.	N.A.	U=2389; p=.07
Negative symptoms	1.4 ± .35	1.5 ± .38	N.A.	N.A.	U=2554; p=.20
Depressive symptoms	1.4 ± .36	1.5 ± .35	N.A.	N.A.	U=2711; p=.56
PANSS scores (UHR: n=49; Patients: n=38)					
Positive symptoms	N.A.	N.A.	12.2 ± 3.2	15.3 ± 5.4	U=628; p=.009
Negative symptoms	N.A.	N.A.	11.4 ± 3.5	14.4 ± 4.7	U=544; p=.001
General symptoms	N.A.	N.A.	26.0 ± 5.4	30.5 ± 8.2	U=624; p=.008

[a] Education according to Verhage (1964); *Abbreviations*: CAPE: Community Assessment of Psychic Experiences; HC: healthy controls; PANSS: Positive and Negative Syndrome Scale; UHR: Ultra-High Risk

effect of group (see supplementary Table S5.1). The post-hoc tests without including covariates also revealed the same group differences, except that the difference between patients and siblings was now also significant (see supplementary Table S5.2).

For the affective alexithymia dimension, the results revealed a significant main effect of group (see Table 5.1) and a significant main effect of education (i.e. individuals with higher levels of education had lower levels of affective alexithymia) ($F_{1,283}$=17.4, p<.001). The main effects of age ($F_{1,283}$=.06, p=.80), gender ($F_{1,283}$=2.7, p=.10) and the group*gender interaction ($F_{3,283}$=.61, p=.61) were not significant. Post-hoc comparisons revealed that the UHR group had significantly lower scores on the affective dimension compared to controls and siblings (see Table 5.2). No other group differences on this dimension were found (see Table 5.2).

|Table 5.2 Post-hoc results (mean difference and p-value) of group differences on the cognitive and affective alexithymia dimension

		Cognitive dimension	Affective dimension
HC	Siblings	-7.0; p=.004*	.9; p=1.0
	UHR	-17.1; p<.001*	7.7; p=.001*
	Patients	-12.9; p<.001*	3.6; p=.59
Siblings	UHR	-10.1; p=.004*	6.7; p=.007*
	Patients	-5.9; p=.39	2.7; p=1.0
UHR	Patients	-4.2; p=1.0	-4.0; p=.75

* Significant at p<.05, corrected for multiple comparisons applying a Bonferroni correction; *Abbreviations*: HC: healthy controls; UHR: Ultra-High Risk

|Table 5.3 Post-hoc results (mean difference and p-value) of group differences on the cognitive alexithymia subscales

		Verbalizing	Identifying	Analyzing
HC	Siblings	-2.1; p=.22	-1.0; p=1.0	-1.9; p=.09
	UHR	-6.1; p<.001*	-6.7; p<.001*	-1.8; p=.61
	Patients	-3.4; p=.16	-5.5; p<.001*	-2.7; p=.16
Siblings	UHR	-4.0; p=.03*	-5.7; p<.001*	.1; p=1.0
	Patients	-1.3; p=1.0	-4.5; p=.001*	-.8; p=1.0
UHR	Patients	2.7; p=.86	1.2; p=1.0	-.9; p=1.0

* Significant at p<.05, corrected for multiple comparisons applying a Bonferroni correction; *Abbreviations*: HC: healthy controls; UHR: Ultra-High Risk

The analyses without including covariates revealed the same group differences (see supplementary Table S5.1 and S5.2).

The alexithymia subscales

To examine whether specific subscales were underlying the group differences on the two alexithymia dimensions, two MANCOVA's were performed. The results of the first MANCOVA showed a main effect of group on the cognitive alexithymia subscales ($F_{9,849}$=6.4; p<.001; Pillai's Trace=.19; partial η^2=.06). Furthermore, a significant main effect of education (i.e. individuals with higher levels of education had lower scores on the cognitive alexithymia subscales) ($F_{3,281}$=4.0; p=.009; Pillai's Trace=.04; partial η^2=.04) and gender (i.e. men had higher scores than women) ($F_{3,281}$=5.5; p=.001; Pillai's Trace=.06; partial η^2=.06) were found, while the main effect of age ($F_{3,281}$=1.1; p=.37; Pillai's Trace=.01; partial η^2=.01) and the group*gender interaction ($F_{9,849}$=1.3; p=.22; Pillai's Trace=.04; partial η^2=.01) were not significant. Follow-up analyses revealed that the groups differed significantly on all three subscales of the cognitive alexithymia dimension (i.e. verbalizing, identifying and analyzing) (see Table 5.1). Post-hoc comparisons are presented in Table 5.3 and show that UHR individuals differed significantly from controls and siblings on the verbalizing scale. Regarding identifying, the UHR and patient group both differed significantly from controls and siblings,

|Table 5.4 Correlations between the two alexithymia dimension and psychotic symptoms

	Cognitive dimension	Affective dimension
CAPE (n=153)		
Positive	ρ=.07; p=.37	ρ=-.20; p=.01
Negative	ρ=.33; p<.001*	ρ=-.26; p=.001*
Depressive	ρ=.15; p=.08	ρ=-.34; p<.001*
PANSS (n=87)		
Positive	ρ=.08; p=.47	ρ=.06; p=.56
Negative	ρ=.05; p=.64	ρ=.17; p=.13
General	ρ=.18; p=.11	ρ=.17; p=.12

*Significant at the corrected p<.008 (Bonferroni correction); *Abbreviations*: CAPE: Community Assessment of Psychic Experiences; PANSS: Positive and Negative Syndrome Scale

but not from each other. On the analyzing scale, the only marginally significant difference was found between the controls and the siblings. The analyses without including covariates revealed nearly the same pattern, except in these analyses patients and UHR individuals also differed significantly from controls on the analyzing subscale (see supplementary Table S5.1 and S5.3).

The second MANCOVA on the two subscales of the affective alexithymia dimension, revealed no significant effect of group ($F_{6,566}$=1.7; p=.12; Pillai's Trace=.04; partial η^2=.02). Furthermore, the main effects of age ($F_{2,282}$=3.8; p=.03; Pillai's Trace=.03; partial η^2=.03), education ($F_{2,282}$=8.2; p<.001; Pillai's Trace=.06; partial η^2=.06) and gender ($F_{2,282}$=25.7; p<.001; Pillai's Trace=.15; partial η^2=.15) were significant (i.e. males with lower levels of education and higher age had higher levels on the affective dimension subscales), while no significant group*gender interaction ($F_{6,566}$=1.5; p=.18; Pillai's Trace=.03; partial η^2=.02) was found. Performing the analysis without including gender, age and education did reveal a significant main effect of group on the affective dimension ($F_{6,578}$=2.3, p=.03; Pillai's Trace=.05; partial η^2=.02). Follow-up analyses revealed a significant effect of group on fantasizing, but not emotionalizing (see supplementary Table S5.1). Post-hoc comparisons showed that only the UHR group had significantly lower scores in the fantasizing subscale compared to controls and siblings (see supplementary Table S5.3).

Correlations between alexithymia and psychopathology

The CAPE was only administered in a subsample of 87 siblings and 66 controls (total n=153, for mean scores see Table 5.1). PANSS data were missing for 3 UHR individuals, which resulted in a total sample of 87 subjects (49 UHR individuals and 38 patients, see Table 5.1 for mean scores). The results showed that affective alexithymia was negatively correlated to subclinical (CAPE) negative and depressive symptoms. Furthermore, the cognitive dimension was positively correlated to the CAPE negative symptoms (see Table 5.4). No significant correlations between the clinical symptoms (PANSS scores) and alexithymia dimensions were found (see Table 5.4).

|DISCUSSION

The aim of the current study was to examine alexithymia in patients with schizophrenia and subjects at increased risk for developing psychosis. The results revealed that siblings of patients with schizophrenia, individuals at UHR for psychosis and patients with schizophrenia show a type-II alexithymia pattern with higher cognitive alexithymia and equal or lower scores on the affective alexithymia dimension compared to controls. Furthermore, there appeared to be a parametric effect of risk on cognitive alexithymia scores with higher risk for schizophrenia being associated with more alexithymia: UHR subjects had higher cognitive alexithymia scores compared to siblings, who in turn scored higher than non-clinical controls. We also found that alexithymia was associated with negative and depressive symptoms in the controls and siblings, but not in the UHR individuals and patients with schizophrenia.

The current results support the idea that alexithymia might be part of the vulnerability for schizophrenia (van 't Wout et al., 2007). Both siblings and subjects at UHR for psychosis showed higher levels of cognitive alexithymia than non-clinical controls. For the UHR group, these results corroborate the earlier published findings in a smaller group (van Rijn et al., 2011). In siblings, van 't Wout et al. (2007) showed a significant gender*group interaction on alexithymia scores, with male siblings showing higher scores on the verbalizing scale compared to male controls. Our results did not show an interaction between group and gender on alexithymia scores, however. Furthermore, the current findings showed that siblings mainly differed on the analyzing subscale. Further research is necessary to examine which particular alexithymia subscales are higher in siblings of patients with alexithymia and to examine the gender*group interaction. However, both the current study and the study of van 't Wout et al. (2007) do point to higher levels of cognitive alexithymia in subjects at genetic risk for psychosis.

In line with our hypothesis, the degree of risk for developing psychosis was associated with the cognitive alexithymia dimension. Subjects with an UHR for psychosis had higher scores on this dimension compared to siblings, who in turn scored higher than controls. Patients also differed from siblings on cognitive alexithymia, but only on the identifying subscale and not on other alexithymia subscales. This finding is agreement with the results of van 't Wout et al. (2007), who also showed that patients only differed from siblings on the identifying subscale. Our study goes beyond the study of van 't Wout et al. (2007) by including larger samples and by including a comparison with UHR subjects. Notably, alexithymia scores did not differ between the UHR group and the patients. This pattern is in agreement with a previous study in which emotion recognition was more impaired in UHR individuals compared to relatives of patients with schizophrenia, while there were no significant differences on emotion recognition between the UHR and the patient group (Kohler et al., 2014). Furthermore, Amminger et al. (2012a) also reported that UHR individuals and schizophrenia patients share the same emotion recognition problems (Amminger et al., 2012a). Taken together, these results suggest that individuals at UHR for psychosis have similar cognitive emotional processing difficulties as patients. Furthermore, these difficulties are also present in siblings with a high genetic risk for psychosis, albeit to a lesser extent.

The current results point to a type-II alexithymia pattern in patients as well as in the two high-risk groups. This type-II alexithymia pattern, with high scores on the cognitive dimension and normal or low scores on the affective dimension, has also previously been reported in patients with schizophrenia (van 't Wout et al., 2007; van der Meer et al., 2009), their siblings (van 't Wout et al., 2007), and subjects at UHR for psychosis (van Rijn et al., 2011). However, the current study is, to the best of our knowledge, the first study to directly compare these

two high-risk groups. It has been suggested that especially this combination of alexithymia scores, awareness of emotional arousal without accompanying emotional cognition, may have negative consequences such as increased negative affect and anxiety (Montebarocci et al., 2006; Moormann et al., 2008a), which are also reported in patients with schizophrenia (Achim et al., 2011; Koreen et al., 1993). Furthermore, this type-II alexithymia pattern appeared to be related to subclinical levels of negative and depressive symptoms. This finding is in line with a previous report in which a type-II alexithymia pattern was related to total schizotypy in a nonclinical sample (Laroi et al., 2008). Furthermore, positive associations between cognitive alexithymia and schizotypy have also been reported (Seghers et al., 2011). Remarkably, no significant associations between clinical symptoms and alexithymia were found in the UHR individuals and patients. Although some previous studies did report associations between symptoms and alexithymia in these groups (Picardi et al., 2012; van 't Wout et al., 2007; van Rijn et al., 2011), the majority of studies did not report any significant associations (Fogley et al., 2014; Henry et al., 2010; Kubota et al., 2011; Kubota et al., 2012; Todarello et al., 2005). Furthermore, alexithymia does not appear to be related to psychotic symptoms in patients with schizophrenia over time (Todarello et al., 2005). It was previously indicated that although patients with schizophrenia often show aberrant levels of affective traits (e.g. neuroticism), these traits are relatively stable in these groups and not related to symptomatology during the phase of the illness (Horan et al., 2008). However, in nonclinical samples, such as controls and relatives, these affective traits do appear to be related to symptomatology (Horan et al., 2008). This in combination with the current findings suggests that a type-II alexithymia pattern might be specifically involved in the vulnerability for psychosis, rather than to psychotic symptoms during the disorder itself. Moreover, a type-II alexithymia pattern might increase the vulnerability of developing psychosis. Future research should examine whether individuals with high levels of type-II alexithymia are indeed at higher risk for developing psychosis through longitudinal research. Furthermore, future studies should elucidate whether training or treatment in an early stage that targets the cognitive dimension of alexithymia, might be beneficial in attenuating subclinical symptoms in groups at high risk for psychosis.

Several limitations of this study should be addressed. First, the groups differed significantly on several demographic variables such as age, level of education and gender. These group differences are due to the fact that the groups are inherently difficult to match as patients with schizophrenia are more likely to be male than female (Aleman et al., 2003) and subjects at UHR are younger because an age below 35 is one of the selection criteria to be considered UHR (Rietdijk et al., 2010). Moreover, subjects at UHR for psychosis and patients with schizophrenia are generally less educated than healthy controls. Controlling for these variables might have had a negative impact on the power of these analyses. However, we also performed the group analyses without controlling for these factors, which revealed almost the same pattern of differences on alexithymia scores between groups. Second, alexithymia was assessed through a self-report measure. Self-report measures rely to a certain extent on the ability to reflect on one's own mental states, a capacity that might be compromised in individuals with alexithymia. Therefore, we recommend future studies to examine alexithymia with self-report and observer-rated measures.

|CONCLUSION

The results indicate that the degree of risk for psychosis is related to higher levels of alexithymia. More specifically, groups at high risk for psychosis, as well as patients, show a type-II alexithymia profile with high levels of cognitive alexithymia and normal or slightly

lower levels of affective alexithymia. Furthermore, alexithymia appeared to be related to nonclinical psychotic symptoms. These findings support the idea that alexithymia, especially the type-II pattern, might be a vulnerability factor for psychosis.

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|SUPPLEMENTARY MATERIAL

These tables represent the results of the MANOVA without including gender as a factor and without age and education as covariates.

|Table S5.1 Test statistics of group differences on the alexithymia dimensions and subscales without including covariates.

	Test statistic
Cognitive dimension	$F_{3,289}$=23.8; p<.001
Verbalizing	$F_{3,289}$=12.0; p<.001
Identifying	$F_{3,289}$=31.8; p<.001
Analyzing	$F_{3,289}$=6.2; p<.001
Affective dimension	$F_{3,289}$=2.7; p=.04
Fantasizing	$F_{3,289}$=4.2; p=.006
Emotionalizing	$F_{3,289}$=.08; p=.97

|Table S5.2 Post-hoc results (mean difference and p-value) of group differences on the cognitive and affective dimension without including covariates

		Cognitive dimension	Affective dimension
HC	Siblings	-6.5; p=.01*	1.0; p=1.0
	UHR	-19.2; p<.001*	4.9; p=.03*
	Patients	-15.9; p<.001*	2.0; p=1.0
Siblings	UHR	-12.8; p<.001*	3.9; p=.19
	Patients	-9.4; p=.008*	.95; p=1.0
UHR	Patients	3.4; p=1.0	-2.9; p=1.0

* Significant at p<.05, corrected for multiple comparisons applying a Bonferroni correction; *Abbreviations*: HC: healthy controls; UHR: Ultra-High Risk

|Table S5.3 Post-hoc results (mean difference and p-value) of group differences on the cognitive alexithymia subscales and the fantasizing subscale without including covariates

		Verbalizing	Identifying	Analyzing	Fantasizing
HC	Siblings	-1.9; p=.40	-.88; p=1.0	-1.7; p=.22	-.66; p=1.0
	UHR	-7.0; p<.001*	-7.5; p<.001*	-3.4; p=.004*	3.5; p=.02*
	Patients	-4.1; p=.02*	-6.5; p<.001*	-3.8; p=.004*	.65; p=1.0
Siblings	UHR	-5.1; p<.001*	-6.6; p<.001*	-1.7; p=.55	4.2; p=.004*
	Patients	-2.3; p=.61	-5.6; p<.001*	-2.1; p=.36	1.3; p=1.0
UHR	Patients	2.9; p=.39	.95; p=1.0	-.42; p=1.0	-2.9; p=.35

* Significant at p<.05, corrected for multiple comparisons applying a Bonferroni correction; *Abbreviations*: HC: healthy controls; UHR: Ultra-High Risk

PART II
SIBLINGS

6 Gray matter, an endophenotype for schizophrenia? A voxel-based morphometry study in siblings of patients with schizophrenia

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|ABSTRACT

BACKGROUND: Gray matter, both volume and concentration, has been proposed as an endophenotype for schizophrenia given a number of reports of gray matter abnormalities in relatives of patients with schizophrenia. However, previous studies on gray matter abnormalities in relatives have produced inconsistent results. The aim of the current study was to examine gray matter differences between controls and siblings of patients with schizophrenia, and to examine whether the age, genetic loading or schizotypy scores of selected individuals could explain the previous reported inconsistencies.

METHODS: To this extent, 89 healthy siblings of patients with schizophrenia and 69 healthy controls, matched for age, gender and education, were compared on gray matter volume and gray matter concentration using voxel-based morphometry (VBM). Furthermore, subsamples were selected based on age (below 30 years old), genetic loading and schizotypy to examine whether this would lead to different results.

RESULTS: The results showed that siblings and controls did not significantly differ on gray matter volume nor concentration. Furthermore, specifically selecting subjects on age, genetic loading or schizotypy did not alter these findings.

CONCLUSION: These results indicate that gray matter measured through VBM might not be a suitable endophenotype for schizophrenia.

|INTRODUCTION

The liability for schizophrenia is heritable (Cardno et al., 1999) with siblings of patients with schizophrenia being at increased risk (around tenfold increase) for developing schizophrenia (Gottesman, 1991). Structural brain abnormalities in patients with schizophrenia have been consistently reported. For example, patients show reduced gray matter (GM) in the frontal, temporal and thalamic regions (for meta-analyses see Fornito et al., 2009; Haijma et al., 2013). Previous studies have suggested that part of these GM abnormalities might not be related to the illness state, but to the genetic risk, and proposed these abnormalities to be an endophenotype for schizophrenia (Moran et al., 2013; Prasad and Keshavan, 2008; Turner et al., 2012). Consistent with this proposal, four recent meta-analyses reported GM reductions in relatives of patients compared to controls (Boos et al., 2007; Cooper et al., 2014; Fusar-Poli et al., 2014b; Palaniyappan et al., 2012). Although the aims of these meta-analyses differed, all meta-analyses compared subjects at genetic high risk with controls. However, the results between these meta-analyses differed substantially and the thresholds applied were rather low ($p<.05$, $p<.005$ and $p<.001$, uncorrected). Two meta-analyses showed GM reductions in relatives compared to controls in the lentiform nucleus (Cooper et al., 2014; Palaniyappan et al., 2012) and medial prefrontal cortex (Palaniyappan et al., 2012), whereas another meta-analysis reported higher levels of GM in the medial prefrontal cortex in siblings (Cooper et al., 2014). Furthermore, reductions in the parahippompal gyrus and anterior cingulate have been reported (Fusar-Poli et al., 2014b), while others reported reductions in the amygdala and hippocampus (Boos et al., 2007). Besides these contradictory reports, the three largest voxel-based morphometry (VBM) studies in siblings of patients (Boos et al., 2012; Honea et al., 2008; Job et al., 2003) did not report any significant differences in whole brain GM between siblings and controls.

In a recent review, several hypotheses were proposed to explain these differences (Moran et al., 2013). The first explanation was that many studies included subjects which already past the critical ages for developing schizophrenia. The onset of schizophrenia typically starts before the age of 30 (Beratis et al., 1994). Siblings who are past this critical age might therefore not be at high risk for schizophrenia anymore, which might reduce the likelihood of finding GM abnormalities in this group. Second, the risk of developing schizophrenia increases as genetic load increases (Keshavan et al., 2005). Relatives from families in which schizophrenia is more common probably share more disease-related genes, which might be associated with larger GM differences. Therefore, including relatives with only one family member with schizophrenia might lead to negative findings while including subjects from multiple affected families, with higher genetic loads, could lead to substantial GM volume differences. Third, although relatives in previous studies were not diagnosed with schizophrenia, differences in subclinical psychotic symptoms might have influenced the results (Moran et al., 2013). Previous studies have related subclinical symptoms to higher (Modinos et al., 2010a) as well as lower GM (Ettinger et al., 2012). Given the idea that the experience of subclinical psychotic symptoms tends to be higher in family members of patients with schizophrenia (Vollema et al., 2002), these symptoms may have confounded previous results. Finally, studies examining GM have applied different techniques. Some examined GM volume (GMV), while others looked at GM concentration (GMC). GMV represents an estimate of the volume of GM, whereas GMC represents the proportion of GM relative to all other tissue types in a region (Taki et al., 2013). Previous research has shown that GMV and GMC results do not necessarily overlap (Taki et al., 2013). Furthermore, a recent meta-analysis reported that in patients with schizophrenia GMC, reductions are larger and more consistent compared to GMV reductions (Fornito et al., 2009). Therefore, it would be of interest to examine whether abovementioned factors have an impact on gray

matter measurements in relatives of patients with schizophrenia.

The aim of the current study was to examine the possible GMV and GMC abnormalities in siblings of patients with schizophrenia and to investigate the impact of age, genetic loading, and subclinical psychotic symptoms on these findings. We therefore performed a VBM analysis on a large group of siblings and controls (n=170) , larger than most GM studies reporting significant differences between relatives and controls (e.g. Hu et al., 2013; Oertel-Knöchel et al., 2012; Tian et al., 2011). Based on previous large VBM studies (Boos et al., 2012; Honea et al., 2008; Job et al., 2003) we did not expect to find GM differences between the general group of siblings and controls. However, we did expect that selecting siblings below the age of 30, with high genetic loading or high schizotypy scores, would result in significant GM abnormalities because of their higher risk profile.

|METHODS

Participants

Structural T1-weighted MRI scans of 95 healthy siblings of patients with schizophrenia and 75 healthy control subjects without first or second degree family members with a psychotic disorder were included. All 95 siblings and 51 healthy controls were included from a multi-center (Groningen and Amsterdam) add-on study from the GROUP project [Genetic Risk & Outcome of Psychosis (Korver et al., 2012)]. Notably, our sample is independent from Boos et al. (2012). An additional number of 24 control subjects were recruited via advertisements in shops and at the university. Participants reported no presence or history of any neurological or psychiatric disorder, which was confirmed with a diagnostic interview. All participants gave written informed consent prior to participation. Furthermore, the study was approved by the local medical ethical committee.

Behavioral measurements

Diagnostic interviews

During the assessment of the GROUP study (max. two years prior to the MRI scan) participants from Groningen were screened with the SCAN interview [Schedules for the Clinical Assessment of Psychiatry (Wing et al., 1990)] and participants from Amsterdam with the CASH [Comprehensive Assessment of Symptoms and History (Andreasen et al., 1992)] to assess the current psychiatric state and psychiatric history of the participants. Participants with an axis-I mood, anxiety or psychotic disorder were excluded from the study. Prior to the MRI session, participants were asked if there were any changes in their psychological well-being since the last GROUP assessment. If participants reported relevant changes in mood, psychotic symptoms or anxiety for which they sought help or received treatment, they were excluded from the study. The additional sample of healthy controls were screened with the SCAN interview prior to the MRI scan.

Community Assessment of Psychic Experiences

The Community Assessment of Psychic Experiences (CAPE) is a 42-item self-report questionnaire used to examine schizotypy (Stefanis et al., 2002). The CAPE measures the frequency of positive, negative, and depressive symptoms on a 4-point scale (0=never; 3=nearly always). Furthermore, when participants report the experience of symptoms (score

of 1 or more on frequency), they are asked how distressed they are by these symptoms (0=not at all; 3=very). Total scores of positive, negative and depressive symptoms are calculated by summing the average frequency and average distress score. The schizotypy total score was calculated by summation of all the averaged frequency and distress scores. Subjects were divided in a high and low schizotypy group by means of a median split on the total schizotypy score. This median split approach is in line with previous studies examining schizotypy (e.g. Nitzburg et al., 2014; van Dongen et al., 2011).

Genetic loading

From all participants of the GROUP study, diagnostic information about family members was assessed with the Family Interview for Genetic Studies (FIGS; Maxwell, 1992). For details on FIGS assessment in the GROUP study see Korver et al. (2012). In the current study the FIGS was used to examine which siblings had at least one other first- or second degree family member with a psychotic disorder, besides their affected sibling. Furthermore, a subsample of healthy controls was selected whom did not report any psychiatric problems in first- or second degree family members.

Image acquisition

Imaging data were acquired using 3.0 Tesla magnetic resonance imaging systems (Philips Intera, Best, NL) located at the University Medical Center Groningen and at the Academic Medical Center in Amsterdam. Both systems were equipped with an 8-SENSE head coil and anatomical images were obtained using a sagittal 3-dimensional T1-weighted sequence (176 slices; TR=9 msec; TE=3.5 msec; FOV=256 mm, voxel size=1 x 1 x 1 mm; slice thickness=1.0 mm).

Statistical analyses

Demographic data were analyzed using SPSS 20 (SPSS Inc, Chicago, Illinois). Two-sample t-tests were calculated to examine possible differences between the controls and siblings on age, education, handedness (as measured with the Edinburgh Handedness Inventory (EHI); Oldfield, 1971), subclinical psychotic symptoms (schizotypy) and total brain volume. Chi-square tests were applied to examine possible group differences on gender and scan site. The same tests were used to examine possible group differences between the subjects from Groningen and Amsterdam on age, education, handedness, total brain volume and gender. Significance was set at $p<.05$, two-sided.

Imaging data were analyzed with unified voxel-based morphometry (VBM) using Statistical Parametric Mapping (SPM8) (http://www.fil.ion.ucl.ac.uk) running under Matlab7 (The MathWorks Inc., Natick, MA, USA). Before processing the data, all images were checked for artifacts and the image origins were manually set at the anterior commissure. Subsequently, images were segmented into gray matter, white matter, and cerebrospinal fluid. The Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) approach was used for optimal registration of individual segments to a group mean template. For the GMV analyses, the GM segments were modulated by the Jacobian determents to correct for volume changes in nonlinear normalization. For the GMC analyses, the GM segments were not modulated. DARTEL normalized modulated and un-modulated GM segments were further normalized to the Montreal Neurological Institute (MNI) space

and smoothed using an 8 mm full width half maximum (FWHM) Gaussian kernel. An 8 mm smoothing kernel is optimal for detecting morphometric differences in both large and small neural structures (Honea et al., 2005; White et al., 2001).

Data were analyzed in the context of a General linear model (GLM). Group was included as a dependent variable and sex, scanner site, age and EHI scores were included as covariates to adjust for their effect on regional brain tissue volumes. Whole brain volume (calculated as the sum of gray and white matter) was entered as a global by means of proportional scaling. To examine the effect of age, an additional GLM was performed including only the participants aged below 30. Furthermore, the high genetic loading sibling group was compared with the low genetic loading control group. To examine the effect of schizotypy on GM, a full factorial model was created with two factors (HC/sibling and high/low schizotypy). Both the GM differences between high and low schizotypy as well as the schizotypy*group interaction (F-test, $p<.001$, $k>20$) were examined. All abovementioned analyses were performed twice, once for GMV and once for GMC. A GM majority optimal threshold mask, created based on the whole sample, was applied to all analyses to eliminate voxels of non-GM (Ridgeway et al., 2009). The abovementioned group comparisons were repeated without including any covariates in the models to examine the possible effect of the covariates on the results. To examine main effects of scanner site, a two-sample t-test was performed between the scanner sites in Groningen and Amsterdam in which whole brain volume was entered as a global by means of proportional scaling.

The threshold for all whole-brain analyses was set at $p<.05$ Familiy-Wise Error (FWE) corrected at the cluster level (corrected for non-stationary of smoothness characteristic for VBM data) with an initial voxel threshold of $p<.001$ (Hayasaka et al., 2004; Woo et al., 2014). Furthermore, region of interest (ROI) analyses were performed in all abovementioned analyses. The ROIs were chosen based on previous reported regions in meta-analyses examining GM in relatives of patients with schizophrenia (Boos et al., 2007; Cooper et al., 2014; Fusar-Poli et al., 2014b; Palaniyappan et al., 2012). The selected ROIs were the amygdala, anterior cingulate, fusiform gyrus, hippocampus, inferior temporal gyrus, insula, lentiform nucleus (consisting of the putamen, pallidum and thalamus), medial frontal gyrus and parahippocampus. The mask were created based on the Automated Anatomical Labeling system implemented in the WFU pickatlas (http://fmri.wfubmc.edu/software/PickAtlas). Results from these ROI analyses had to meet $p<.009$ FWE corrected for the spatial extent of the ROI to be considered significant. This $p<.009$ was chosen to correct for the number of ROIs (i.e. 9), while taking into account their non-independency of the dependent measure [i.e. total GM volume of AAL masks: mean correlation $r=.23$, corrected for total brain volume (http://www.quantativeskills.com/sisa/index.htm)].

|RESULTS

Demographic and behavioral results

The healthy controls and siblings did not differ significantly on gender, age, education, and handedness (see Table 6.1). Furthermore, no differences were found between the two groups on subclinical psychotic symptoms as measured with the CAPE (see Table 6.1). No significant differences on gender, age, education and handedness were found between the subjects from Groningen and the subjects from Amsterdam (see Table 6.2).

|Table 6.1 Mean, standard deviations and group differences between healthy controls and siblings on demographic variables, total brain volume and schizotypy

	HC (n=69)	Siblings (n=89)	Test statistic	
Gender (% male)	38 (55%)	41 (46%)	χ^2 = 1.26	p = .26
Age (in years)	33.5 ± 10.2	32.1 ± 8.1	t = .93	p = .36
Education[a]	6.1 ± 0.8	5.9 ± 0.8	t = 1.23	p = .22
Scan site (% Amsterdam)	28 (41%)	44 (50%)	χ^2 = 1.23	p = .27
Handedness				
% right	87%	82%		
EHI	65.3 ± 48.6	60.6 ± 52.8	t = .57	p = .57
Total brain volume	986 ± 65	982 ± 63	t = .33	p = .74
CAPE (based on subsample of 63 HC and 82 siblings)				
Positive symptoms	.33 ± .54	.40 ± .61	t =-.70	p = .48
Negative symptoms	1.03 ± .85	1.15 ± .86	t =-.82	p = .41
Depressive symptoms	1.30 ± .93	1.35 ± .88	t =-.31	p = .76
Total score	2.70 ± 1.97	2.90 ± 1.99	t =-.59	p = .56

[a] Scoring according to Verhage, 1964; *Abbreviations*: CAPE: Community Assessment of Psychic Experiences; EHI: Edinburgh handedness inventory; HC: healthy controls

VBM-results

Eight subjects (4 controls and 4 siblings) were excluded because of poor data quality. Furthermore, three subjects (2 controls and 1 sibling) were excluded because they were identified as outliers by the homogeneity check (VBM8 toolbox version 435, http://dbm.neur.uni-jena.de/vbm). The final sample therefore consisted of 69 controls and 89 siblings (for demographic details of this final sample see Table 6.1). No significant differences in total brain volume were found between the two groups (see Table 6.1) nor the two scanner sites (see Table 6.2).

The VBM results revealed no significant regional differences in GMV nor GMC between the siblings and healthy controls in the whole brain analyses. Furthermore, no significant differences were found in the selected ROIs, except for a higher ACC volume in siblings compared to controls [p=.012; k=429; Z=3.72; 8,33,15 (x,y,z)]. However, this finding did not survive the multiple comparison threshold (p<.009). No GMV nor GMC differences were found between the scans made in Groningen and the scans made in Amsterdam.

Age < 30

Selecting participants aged below 30 resulted in 33 controls and 40 siblings. The two groups did not differ on gender, age, education, handedness (EHI), scanner site and CAPE scores (lowest p=.21). The VBM results did not reveal any significant group differences on GMV nor GMC in the whole brain or the ROI analyses.

|Table 6.2 Mean, standard deviations and group differences between the subjects from the two scanner sites on demographic variables and total brain volume

	HC (n=69)			Siblings (n=89)		
	Groningen (n=41)	Amsterdam (n=28)	Test statistic	Groningen (n=45)	Amsterdam (n=44)	Test statistic
Gender (% male)	21 (51%)	17 (61%)	χ^2=.61, p=.47	22 (49%)	19 (43%)	χ^2=.29, p=.59
Age (in years)	33.7 ± 10.7	33.2 ± 9.6	t=.20, p=.85	30.9 ± 7.9	33.2 ± 8.3	t=-1.3, p=.19
Education[a]	6.0 ± .7	6.1 ± .8	t=-.44, p=.66	5.8 ± .8	6.0 ± .9	t=-.87, p=.39
Handedness						
EHI	68.0 ± 44.9	61.3 ± 54.2	t=.56, p=.58	63.1 ± 46.5	58.1 ± 59.0	t=.45, p=.65
TBV	986 ± 59	984 ± 75	t=.15, p=.88	981 ± 58	983 ± 69	t=-.14, p=.89

[a] Scoring according to Verhage, 1964; *Abbreviations*: EHI: Edinburgh Handedness Inventory; HC: healthy controls; TBV: total brain volume

Genetic loading

Selecting siblings with at least one additional family member with a psychotic disorder in first- or second-degree family members, resulted in 20 siblings. These 20 siblings were compared with 21 healthy controls with no reports of any psychiatric problems in first- or second degree family members. The groups did not differ on gender, age, education, EHI, and scanner site (lowest p=.11). However, there was a small, but not significant, difference in the CAPE positive symptoms scores (p=.08) with higher scores in the sibling group ($M_{controls}$=.27; $M_{siblings}$=.61). The VBM results did not show significant GMV or GMC differences between the high genetic risk siblings and the low genetic risk controls in the whole brain or the ROI analyses.

Schizotypy

Due to missing or incomplete CAPE data, six controls and seven siblings had to be excluded from this analysis, leaving 63 controls and 82 siblings not differing on demographic variables (lowest p=.25). Furthermore, siblings and healthy controls did not differ in CAPE scores (see Table 6.1). To divide the groups in high and low schizotypy, a median-split based on total CAPE scores was performed per group ($Median_{controls}$=2.4; $Median_{siblings}$=2.4). The resulting high schizotypy group (n=72) did not differ from the low schizotypy group (n=73) on gender, age, education, EHI, and scanner site (lowest p=.43). As provided, these two groups did differ on CAPE positive symptoms (M_{high}=.59; M_{low}=.13; p<.001), negative symptoms (M_{high}=1.7; M_{low}=.49; p<.001), depressive symptoms (M_{high}=1.9; M_{low}=.67; p<.001), and CAPE total scores (M_{high}=4.2; M_{low}=1.3; p<.001). The full-factorial analysis revealed that high schizotypal individuals had larger right precuneus/posterior cingulate volume compared to low schizotypal individuals [p=.03; k=730; Z=4.45; 6,-40,48 (x,y,z)], but no differences were found in GMC. Furthermore, the interaction analysis did not reveal a schizotypy*group interaction on GMV nor GMC in the whole brain or the ROI analyses (F-test, k<20 on p<.001).

Repeating abovementioned analyses without including the covariates (sex, scanner site, age and handedness) did not significantly alter the findings.

|DISCUSSION

The aim of the current study was to examine putative GM differences between siblings of patients with schizophrenia and controls and to investigate whether previously suggested factors as age, genetic loading and schizotypy influence these GM differences. The results revealed no significant differences in GMV nor GMC between siblings and controls. Furthermore, selecting specifically on age, genetic loading or schizotypy did not alter these findings, although there was an effect for schizotypy across groups.

The finding of non-significant GM differences between siblings and controls is in accordance with three previous large studies on GM in relatives of patients with schizophrenia (Boos et al., 2012; Honea et al., 2008; Job et al., 2003). This suggests that enhanced genetic risk for developing schizophrenia might not be related to substantial differences in GM. Although large studies are unable to find GM alterations in relatives of patients with schizophrenia, smaller studies with lower thresholds often do report GM differences between relatives and controls (e.g. Lui et al., 2009; Oertel-Knöchel et al., 2012) which may explain the positive (albeit inconsistent) effects found in previous meta-analyses (Boos et al., 2007; Fusar-Poli et al., 2014b; Palaniyappan et al., 2012). These discrepancies were previously proposed to be due to differences in age, genetic loading, and schizotypy (Moran et al., 2013). However, the current results indicate that these factors might not explain these discrepancies.

The results revealed that selecting young participants, below the critical ages of developing schizophrenia (<30), did not result in GM differences between controls and siblings, indicating that the null finding in the total sample was not caused by including siblings above the age of 30. This finding is consistent with a previous study in which including subjects ages below 30 also did not result in significant GM differences between controls and relatives of schizophrenia (Job et al., 2003).

Previous studies have examined subjects at high genetic risk for schizophrenia. However, this is as far as we know, the first VBM study comparing these high genetic risk individuals (e.g. one affected sibling and at least one other affected first- or second degree family member) to low risk controls (i.e. no reported psychiatric problems in first- or second degree family members). The results showed that even when comparing a high genetic risk group of siblings and a low genetic risk group of controls, no GMV nor GMC differences were found. This finding is in line with several studies unable to find GM differences in unaffected monozygotic twins of patients with schizophrenia (Borgwardt et al., 2010; van Haren et al., 2004), which are at the highest possible genetic risk for developing schizophrenia.

Examining GM differences between high and low schizotypal individuals (irrespective of group) revealed larger GMV (but not GMC) in the right precuneus which is in accordance with a previous study on schizotypy in healthy undergraduates (Modinos et al., 2010a). This larger precuneus volume might be underlying the problems in the reallocation of attention as suggested in a recent fMRI study in which less deactivation of the right precuneus was found in schizotypy (Fink et al., 2013). As suggested by the authors, this process could result in overinclusive thinking which is a characteristic of schizophrenia (Cutting et al., 1987). Although larger precuneus volume was found in the high schizotypy group, no interaction between schizotypy and group (HC or sibling) on GM was found. These results indicate that previous reports on larger precuneus volume in relatives of patients with schizophrenia (Honea et al., 2008) (after lowering threshold for exploratory purposes) might be caused by differences on schizotypy scores rather than genetic risk differences between groups. The fact that GMV differences are associated with subclinical psychotic symptoms but not to

genetic risk indicates that volume differences indeed might be more related to psychotic symptoms, as previous proposed by Boos et al. (2012).

We are not certain whether the affected siblings of our genetic risk group had GM abnormalities, as this was not investigated. However, previous reports reliably documented GM abnormalities in patients with schizophrenia (Fornito et al., 2009; Glahn et al., 2008). Furthermore, in a study of Boos et al. (2007) GM abnormalities in patients were reported, while their non-affected siblings did not show these abnormalities.

This study indicates that GM measured through VBM might not be a suitable endophenotype for schizophrenia. One important aspect of an endophenotype is that it should be present in unaffected relatives of patients. The current study combined with previous reports (Boos et al., 2012; Honea et al., 2008; Job et al., 2003) questions the idea that gray matter abnormalities are present in relatives of patients with schizophrenia. However, the current results did reveal marginal significant higher anterior cingulate cortex (ACC) volume in siblings of patients with schizophrenia through a ROI-analysis. This finding is inconsistent with some previous reports on lower ACC volume in relatives (Honea et al., 2008; Job et al., 2003). However, these studies also only found lower ACC volume when lowering the threshold or when performing ROI analyses. Furthermore, others have failed to show any volumetric differences in the ACC (e.g. Hu et al., 2013; McIntosh et al., 2004). The lack of reproducibility of these and other previous reported findings, as indicated by the different results in four separate meta-analyses (Boos et al., 2007; Fusar-Poli et al., 2014; Palaniyappan et al., 2012), rises further doubts to the value of GM as an endophenotype for schizophrenia. For example, only one of these meta-analyses reported lower ACC volume in siblings of patients with schizophrenia (Fusar-Poli et al., 2014b). These divergent findings may not be fully explained by differences in age, schizotypy and genetic loading, because specifically selecting participants on these factors did not reveal any GM differences between siblings and controls. One possibility could be that only specific schizophrenia related genes are associated with GM abnormalities, such as aberrant ACC volume. For example, previous research has shown that DISC-1 risk allele carriers have lower GMV in the ACC (Szeszko et al., 2008), while CNNM2 risk allele carriers have higher ACC volume (Rose et al., 2014). Future research should examine whether these genetic variations can explain the divergent findings on GM abnormalities in relatives of patients with schizophrenia.

Limitations

Several limitations of the current study need to be addressed. First, by subdividing the current sample, the group sizes for these sub-analyses became smaller. Especially, the analysis regarding the genetic loading has lower power compared to the other analyses. However, the power of this analysis is still sufficient to detect medium to small effects (Friston et al., 1996). Furthermore, selecting on high genetic loading versus low genetic loading increased the sensitivity to detect differences related to genetic risk. Hence, smaller sample sizes would be sufficient to detect GM differences between these groups. Second, the group was too small to select subjects on two factors combined (e.g. aged below 30 and high genetic risk). Future research should consider selecting subjects specifically on the combination of these factors to examine whether this has an effect on gray matter. Third, participants were scanned using two different scanners. Although the reliability of multi-scanner VBM has proven to be good when adding scanner as a covariate (Focke et al., 2011; Stonnington et al., 2008), including scan site as a covariate may have lowered the statistical power of detecting between group differences. Therefore, we repeated the analyses without

including any covariates, which did not significantly change the results. Fourth, all participants with an axis-I mood, anxiety or psychotic disorder were excluded from this study. This exclusion method may have resulted in excluding the most vulnerable siblings. However, this method was chosen to make sure that possible gray matter abnormalities were not due to comorbid psychiatric disorders. The current findings show that the differences between previous reported findings might not be explained by differences in age, genetic loading or schizotypy between studies, nor by examining either GMV or GMC. However, it is still possible that methodological differences (e.g. differences in T1 acquisition, the applied VBM method or correction for total brain volume) may explain these divergent findings in the literature. We therefore encourage future research on the possible influence of these methodological differences.

|CONCLUSION

The current study provides further support for the hypothesis that GM as measured with VBM might not be an endophenotype for schizophrenia and that it might be more related to the illness itself. Future research should focus more on brain connectivity and functional neuroimaging as possible endophenotypes, as these seem to differ more consistently across unaffected relatives (MacDonald et al., 2009; Pettersson-Yeo et al., 2011). Furthermore, research should examine the role of specific genetic variations on gray matter, specifically on the anterior cingulate cortex.

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7 Emotion regulation in siblings of patients with schizophrenia: A functional magnetic resonance imaging study

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|ABSTRACT

BACKGROUND: Patients with schizophrenia show difficulties with the regulation of emotions. These difficulties are reflected by lower prefrontal activation during reappraisal and less successful down-regulation of negative affect. Recently, it was suggested that these emotion regulation difficulties might already occur before the onset of psychosis. Therefore, the aim of the current study was to examine whether subjects at increased genetic risk for schizophrenia indeed already show abnormalities in the use and neural correlates of emotion regulation.

METHODS: To this extent, we investigated two emotion regulation strategies (reappraisal and suppression) in 72 siblings of patients with schizophrenia and 66 matched controls during an fMRI-task.

RESULTS: The results revealed no differences in brain activation during the application of reappraisal or suppression. Furthermore, siblings were equally capable as controls to regulate their negative affect.

CONCLUSION: These results indicate that merely being a relative of a patient with schizophrenia may not affect the ability to regulate negative emotions. Future research should investigate the association with genetic risk scores in relatives, as some relatives have a larger number or risk gene variants than others.

|INTRODUCTION

Patients with schizophrenia may have difficulties with the processing and regulation of emotions. For example, behavioral studies have shown that patients are impaired at emotion recognition (Kohler et al., 2010), attribute salience to non-salient stimuli (Cohen and Minor, 2010) and apply less efficient emotion regulation strategies (van der Meer et al., 2009). Emotion regulation can be defined as the way we change the experience and expression of emotions (Gross, 1998). Two often applied emotion regulation strategies are reappraisal and suppression. Reappraisal is the reinterpretation of a stimulus in such a way that it becomes less emotional disturbing (e.g. decreasing the negative valence of a picture of a car crash by thinking nobody got hurt). Suppression is the inhibition of emotional expressive behavior by not showing how you feel (e.g. keeping a poker face) (Gross, 1998). The use of reappraisal is associated with good social functioning and positive affect, while the use of suppression is associated with poor social functioning and negative affect (Gross, 2002). Several studies reported that patients with schizophrenia use less reappraisal and more suppression compared to controls (Kimhy et al., 2012; Livingstone et al., 2009; van der Meer et al., 2009). Furthermore, neuroimaging research has indicated that the neural basis underlying reappraisal is impaired in schizophrenia (Morris et al., 2012; van der Meer et al., 2014).

In healthy controls, activation increases during reappraisal in the ventrolateral prefrontal cortex (VLPFC), dorsolateral prefrontal cortex (DLPFC), dorsomedial prefrontal cortex (DMPFC) and the temporal cortex (Buhle et al., 2013; Diekhof et al., 2011). Subsequently, activation in the amygdala decreases, resulting in lower negative affect (Diekhof et al., 2011). Patients with schizophrenia show less increased prefrontal activation during reappraisal (Morris et al., 2012; van der Meer et al., 2014) and are, as a result, less capable of down-regulating negative affect through reappraisal (Morris et al., 2012). Suppression, on the other hand, is characterized by higher activation in regions involved in motor control, such as the DLPFC, insula, precentral gyrus, supplementary motor area and supramarginal gyrus (Hayes et al., 2010; Vanderhasselt et al., 2012; Vrticka et al., 2011). As far as we know, no studies have yet examined the neural basis of suppression in schizophrenia.

Longitudinal research has indicated that emotion dysregulation might already precede the onset of psychosis (Fowler et al., 2012; Smeets et al., 2012). If so, one would expect emotion regulation difficulties already to be present in subjects at increased risk for developing schizophrenia. Siblings of patients with schizophrenia are at a tenfold increased genetic risk for developing psychosis (Gottesman, 1991) and may have similar emotion processing difficulties as patients, albeit to a lesser extent. For example, siblings perform worse at emotion recognition tasks compared to controls without a history of psychosis in the family (Lavoie et al., 2013). Recently, a study from our group showed that during reappraisal, activation in the VLPFC and superior temporal gyrus was lower in siblings compared to controls (van der Meer et al., 2014). This lower activation may indicate some emotion regulation difficulties in the sibling group. However, despite this lower activation pattern, siblings were equally capable of down-regulating negative affect through reappraisal as controls. Furthermore, siblings reported to apply reappraisal and suppression to the same extent as controls (van der Meer et al., 2014). One of the possible explanations for this discrepancy between brain activation and behavioral measures could be the modest sample size of the study, which might have lowered the power to detect behavioral differences. Besides this single study on the neural correlates of reappraisal in siblings of patients with schizophrenia, no other studies have yet examined emotion regulation in individuals at high genetic risk for developing schizophrenia. Furthermore, the neural basis of suppression has not yet been examined in this group.

Therefore, the aim of the current study was to further examine whether siblings differ from controls in the use of emotion regulation and the underlying neural correlates. To this extent we measured brain activation through functional magnetic resonance imaging (fMRI) during reappraisal and suppression in a large sample of siblings of patients with schizophrenia and matched controls (n=138). We expected to find lower activation in the VLPFC and temporal cortex in siblings, as was previously reported (van der Meer et al., 2014). Furthermore, we expected siblings to be less capable of down-regulating negative affect through reappraisal. The study regarding suppression was exploratory as this was the first study on the neural basis of suppression in subjects at high risk for schizophrenia.

|METHODS

Participants

Eighty siblings of patients with schizophrenia and 70 matched healthy controls without any first- or second-degree family members with a psychotic disorder were included in this study. All 80 siblings and 56 controls were included from a multi-center (Groningen and Amsterdam) add-on study from the GROUP project [Genetic Risk & Outcome of Psychosis; (Korver et al., 2012)]. This sample partially overlaps with a previous study from our group [$n_{siblings}$=20, $n_{controls}$=8 (van der Meer et al., 2014)]. The other 24 controls were recruited outside of the GROUP study through advertisements. None of the participants reported a presence or history of any psychiatric or neurological disorder. The study was approved by the local medical ethical committee and conducted according to the declaration of Helsinki. Demographic details of the final sample (72 siblings and 66 controls; for reasons of exclusion see results section) are presented in Table 7.1.

Behavioral measurements

Diagnostic interviews

During the assessment of the GROUP study (max. two years prior to the fMRI scan) participants from Groningen were screened with the SCAN interview [Schedules for the Clinical Assessment of Psychiatry (Wing et al., 1990)] and participants from Amsterdam with the CASH [Comprehensive Assessment of Symptoms and History (Andreasen et al., 1992)] to assess the psychiatric state and history of the participants. When participants were diagnosed with a clinical disorder, they were excluded from the study. Prior to the fMRI-scan, participants were asked whether they experienced any significant changes in their psychological well-being since the last GROUP assessment. If so, participants were excluded from the study. The additional sample of 24 healthy controls were screened with the SCAN interview prior to the fMRI-scan.

Emotion Regulation Questionnaire

The Emotion Regulation Questionnaire (ERQ) was administered to examine the reported use of reappraisal and suppression (Gross and John, 2003). Subjects had to rate on a 7-point scale to what extent a certain statement applied to them (1=strongly disagree; 7=strongly agree). The ERQ consists of ten items of which six examine reappraisal and four examine suppression. To get a clear view on the relationship between the two emotion regulation strategies, the average score per subscales was calculated. The ERQ is a reliable and valid measure of emotion regulation (Gross and John, 2003).

Table 7.1 Mean, standard deviation and between-group test statistics on demographics, affect and emotion regulation

	Healthy controls (n=66)	Siblings (n=72)	Test statistic
Demographics			
Gender (% male)	48	47	χ^2=.02, p=.88
Age (in years)	32.5 ± 10.5	31.8 ± 7.8	t=.45, p=.65
Education[a]	6.1 ± .7	5.9 ± .8	t=.90, p=.37
Scan site (% Groningen)	60	50	χ^2=1.57, p=.21
Handedness (EHI)	65.7 ± 46.0	57.8 ± 54.7	t=.91, p=.37
PANAS			
Positive affect	31.4 ± 7.5	32.8 ± 5.6	t=-1.2, p=.24
Negative affect	13.4 ± 5.3	14.3 ± 3.8	t=-1.1, p=.27
ERQ			
Reappraisal	5.0 ±.9	5.0 ± 1.1	t=-.01, p=.99
Suppression	2.9 ± 1.2	3.2 ± 1.1	t=-1.3, p=.20
Rating scores			
Attend neutral	1.1 ± .2	1.2 ± .2	U=2086.5, p=.21
Attend negative	2.6 ± .6	2.7 ± .5	U=2225.0, p=.52
Reappraise	2.2 ± .6	2.1 ± .5	U=2289.0, p=.71
Suppress	2.5 ± .6	2.5 ± .5	U=2310.5, p=.78

[a] Level of education according to Verhage, 1964; *Abbreviations*: EHI: Edinburgh Handedness Inventory; ERQ: Emotion Regulation Questionnaire; PANAS: Positive and Negative Affect Scale

Positive and Negative Affect Scale

The positive and negative affect scale [PANAS (Watson et al., 1988)] was used to measure the current affective state of the participants. The PANAS has been proven to be a reliable and valid measure of positive and negative affect (Crawford and Henry, 2004), which consists of 10 positive and 10 negative affect items. Participants had to rate on a five-point scale to what extent they experienced certain mood states prior to the fMRI-scan.

Community Assessment of Psychic Experiences

The community assessment of psychic experiences (CAPE) is a 42-item self-report questionnaire which was applied to examine schizotypy (Stefanis et al., 2002). The frequency of positive, negative and depressive symptoms was measured on a four-point scale (0=never; 3=nearly always). Furthermore, when participants reported the experience of symptoms (score ≥1 on frequency), they were asked how distressed they were by these symptoms (0=not at all; 3=very). Total scores of positive, negative and depressive symptoms were calculated by summing the average frequency and average distress score.

Emotion Regulation Task

The emotion regulation task [adapted from (Ochsner and Gross, 2005)] consisted of four conditions, Attend Neutral, Attend Negative, Reappraise and Suppress. Sixty-six negative (mean valence: 2.54, mean arousal: 5.83) and 22 neutral pictures (mean valence: 5.10, mean arousal: 3.26) from the International Affective Picture System (IAPS) were used in the task. Each trial was constructed as follows. First, a picture with the instruction 'view' appeared (View condition, 2s). Subsequently, the instruction 'view' changed in either 'reappraise', 'suppress' or 'attend' (Regulation condition, 4s). Reappraise instructed the participants to reappraise the picture in such a way that it became less emotionally disturbing. Suppression instructed the participants to refrain from expressing their emotions in such a way that bystanders would not be able to read the emotional facial expressions of the subject. During attend, participants just had to look closely at the picture and not change the way they were feeling. The 22 neutral pictures were always paired with the 'attend' instruction. Negative pictures could be paired with either reappraise (22 pictures), suppress (22 pictures) or attend (22 pictures). After regulation, a black screen appeared (Lingering, 2s). Subsequently, participants were asked to rate how negative they were feeling on a four-point scale (1=not negative at all; 4=extremely negative) (Rating, 3s). After rating, the word 'relax' appeared (4s) followed by a black screen (0.5s) to alert participants the next trial was coming. A single trial lasted for 15.5 seconds. After 9 or 10 trials, a fixation cross appeared for 20 seconds.

Prior to the fMRI scan, a short training was given to teach the application of reappraisal and suppression strategies. During this training, participants practiced the different strategies on negative pictures by telling the researches how they would apply the reappraisal or suppression strategy.

Image acquisition

MRI data was acquired using two 3.0 Tesla whole body scanners (Philips Intera, Best, NL) located at the University Medical Center Groningen and the Academic Medical Center in Amsterdam. Both systems were equipped with an 8-SENSE head coil and scan parameters were set identical. The functional images were acquired by a T2-weighted echo producing 37 slices of 3.5 mm thick with no gap. The images were slightly tilted (30 degrees) to prevent artifacts due to nasal cavities. The functional scans were made in the axial plane (TR=2s; TE=30s; flip angle (α)=70°; FOV=224.0, 129.5, 224.0; in-plane resolution 64x62 pixels; isotropic voxels of 3.5 mm) and were scanned interleaved. The T1-weighted anatomical image (170 slices; isotropic voxels of 1 mm; TR=9 ms; TE=3.54 ms; α=8°; FOV=256 mm) was acquired in the bicommissural plane, covering the whole brain.

Statistical analyses

Behavioral analyses were performed using SPSS 20 (SPSS Inc., Chicago, IL, USA). Two-sample t-tests were conducted to examine the possible differences between siblings and controls on age, education, handedness [as measured with the Edinburgh Handedness Inventory (EHI) (Oldfield, 1971)], ERQ, PANAS and CAPE scores. Chi-square tests were applied to examine possible group differences on gender and scan site. The significance level was set at $p<.05$, two-sided. Friedman's ANOVA was applied to examine the main effect of condition on negative affect during the emotion regulation task, due to non-normality of the rating scores ($p<.05$). The Wilcoxon signed-rank test was used for the post-hoc analyses. To examine

possible differences between siblings and controls on the rating scores, Mann-Whitney U tests were performed. For both the post-hoc and between group analyses, the significance level was set at p<.017 to correct for multiple testing (Bonferroni correction for 3 tests).

The fMRI data was analyzed using Statistical Parametric Mapping (SPM 8) (www.fil.ion.ucl.ac.uk) using Matlab 7 (The MathWorks Inc., Natick, MA, USA). Before processing the data, all images were checked for artifacts. Slice timing was applied to the functional images and the functional data were spatially realigned, resliced and coregistered. The anatomical data were segmented. To enhance the accuracy of inter-subject alignment, the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) approach was used to create a gray matter template based on the gray matter segmented images of all subjects. This created template was used to normalize the functional images to and affine-transform them into Montreal Neurological Institute (MNI) stereotactic space. Data was smoothed with a full-width half-maximum Gaussian kernel of 6 mm. Subjects with excessive head motion or head motion correlated to the task paradigm were excluded from the study.

Sixteen task-related regressors were modeled with a boxcar function convolving a hemodynamic response function. The regressors View and Relax were divided into View/Relax neutral and View/Relax negative. The other regressors, Regulation, Lingering and Rating were subdivided into a Reappraise, Suppress, Attend Negative and Attend Neutral part. Additionally, the realignment parameters and the first derivatives thereof were entered as covariates to correct for the effects related to head motion. Five contrasts were made for each participant: 1) View Neutral versus Baseline; 2) View Negative versus View Neutral; 3) Attend Negative versus Attend Neutral; 4) Reappraise versus Attend Negative; 5) Suppress versus Attend Negative.

To examine task-related activation, one sample t-tests were conducted. Sex, handedness (EHI) and scanner site (Amsterdam vs. Groningen) were entered as covariates of no interest. To examine brain activation differences between siblings and controls, two-sample t-tests were conducted including the covariates mentioned above. To limit possible false positives due to multiple comparisons, effects had to meet p<.05 Family-Wise Error (FWE) corrected at the cluster level to be considered statistically significant with an initial height-threshold of p<.001 for all the analyses. Because of specific hypotheses regarding the amygdala, a Small Volume Correction (SVC) with a p<.05 cluster correction was applied if this region would not show in the whole-brain analyses. Furthermore, abovementioned two-sample t-tests were repeated without including covariates, to examine the possible influences of the included covariates on the results.

|RESULTS

Behavioral results

Four healthy controls and eight siblings had to be excluded from the analyses due to extensive movement during the task (n=7), incomplete fMRI data (n=3) or missing task data caused by technical difficulties (n=2). The final sample therefore consisted of 66 controls and 72 siblings. These two groups did not significantly differ on gender, age, education, handedness and scanner site (see Table 7.1). Furthermore, the groups did not differ on positive or negative affect prior to scanning nor on the reported use of reappraisal and suppression (see Table 7.1). Schizotypy scores (measured with the CAPE) were only administered in a subsample of 68 siblings and 57 controls. No significant differences on positive ($M_{controls}$=.37; $M_{siblings}$=.43; t=-.53; p=.60), negative ($M_{controls}$=1.1; $M_{siblings}$=1.1; t=-.15;

p=.88) or depressive symptoms ($M_{controls}$=1.4; $M_{siblings}$=1.3; t=.24; p=.81) were found between siblings and controls.

A significant main effect of condition (Attend neutral, Attend negative, Reappraise, Suppress) was observed on the rating scores of the emotion regulation task ($\chi^2(3)=320.5$, p<.001). Post hoc analyses revealed that subjects rated the negative pictures as more negative than the neutral pictures (Z=10.1, p<.001). Furthermore, subjects were able to reduce their negative affect through reappraisal (Z=-9.3, p<.001) and suppression (Z=-4.0, p<.001). Healthy controls and siblings did not significantly differ on the rating scores of the emotion regulation task (see Table 7.1).

Neuroimaging results

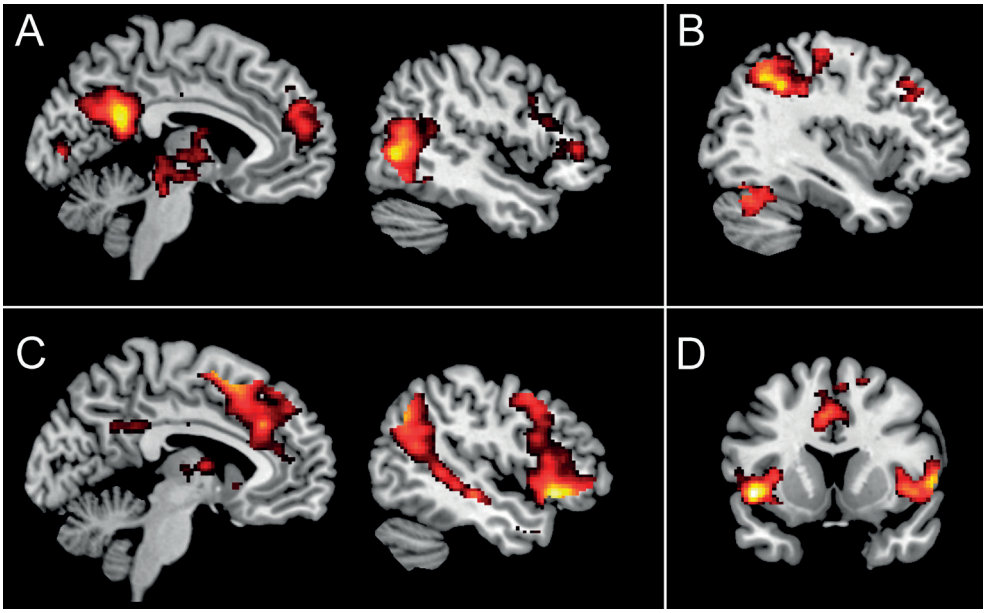
Healthy controls and siblings did not differ on brain activation in response to neutral picture viewing. The first two seconds of viewing a negative picture compared to viewing a neutral picture resulted in the activation of a widespread emotion processing network. Both the healthy controls and siblings activated the bilateral middle temporal gyrus, bilateral precuneus, bilateral inferior frontal gyrus and the bilateral medial superior frontal gyrus (Figure 7.1A, for full details see supplementary Table S7.1). Furthermore, the healthy controls activated the left [p=.01, k=21, Z=4.84; -16,-2,-12(x,y,z)] and right amygdala [p=.002, k=64, Z=4.40; 22,-4,-14 (x,y,z)] during negative picture viewing compared to neutral picture viewing. The activation of the amygdala in the siblings group did not reach the significance level [p=.07, k=1, Z=3.10; 22,-6,-12 (x,y,z)]. However, no significant differences in amygdala activation or activation in other regions between healthy controls and siblings were found for the contrast view negative versus view neutral.

The four seconds of attending a negative picture (after the first two seconds of viewing) resulted in higher activation in regions such as the precuneus, middle frontal gyrus and parietal regions in both siblings and controls (Figure 7.1B and supplementary Table S7.1). No significant differences in brain activation were found between controls and siblings.

The contrast reappraising compared to attending negative pictures revealed higher activation in regions of the emotion regulation network such as, the bilateral VLPFC, the left DLPFC, the DMPFC and the bilateral middle temporal gyrus (Figure 7.1C and supplementary Table S7.1) in both controls and siblings. No significant activation decreases were found. Furthermore, no significant activation differences were found between controls and siblings.

Suppressing negative pictures compared to attending resulted in higher activation in, amongst others, the bilateral insula, supplementary motor cortex and supramarginal gyrus. Furthermore, activation was lower in occipital regions and the postcentral gyrus during suppression. These results were found in both controls as well as siblings (Figure 7.1D and supplementary Table 7.1). The results revealed no significant differences between siblings and controls.

All abovementioned group comparisons were repeated without covariates. This did not significantly alter the reported findings (i.e. no significant differences in any of the contrasts were found).



|Fig 7.1 Main effects of negative emotion processing and emotion regulation in healthy controls. (A) Increased activation during negative picture viewing compared with neutral picture viewing. (B) Increased activation during attending a negative picture compared with a neutral picture. (C) Increased activation during the reappraisal of a negative picture compared to attending. (D) Increased activation during the suppression of a negative picture compared with attending. Results are displayed at p<.001, with a p<.05 FWE cluster correction. The results are overlaid on a MNI template brain.

|DISCUSSION

The aim of the current study was to examine whether controls and siblings of patients with schizophrenia differ in the use of reappraisal and suppression and the underlying brain activation. The results revealed no significant differences between the groups on brain activation during negative picture viewing, reappraisal or suppression. Furthermore, siblings did not differ from controls on the reported use of emotion regulation strategies nor the capacity to down-regulate negative affect through reappraisal or suppression.

The processing of negative emotional pictures resulted in the activation of a widespread emotion processing network, including the amygdala, middle temporal gyrus and medial frontal gyrus (Phan et al., 2002). No differences in brain activation were found between siblings and controls during negative emotion picture viewing. This lack of differences is in line with a previous study in which adolescent offspring from patients with schizophrenia did not differ from controls on brain activation during the viewing of negative facial expressions (Barbour et al., 2012). Other neuroimaging studies on negative emotion processing in relatives of patients with schizophrenia revealed higher activation in the frontal cortex and precentral gyrus (Li et al., 2012) and in the amygdala, medial prefrontal cortex, cingulate cortex and middle temporal gyrus (van Buuren et al., 2011) compared to controls. However, lower activation in these regions has also been reported in this group (Lo Bianco et al., 2013; Venkatasubramanian et al., 2010). Given this lack of consistent findings, further research is needed to disentangle these differences between studies. A possible explanation for the divergent findings could be that siblings may have differed in schizotypy scores between

studies. Previous research has shown that schizotypy is related to differential brain activation patterns during emotion processing (Modinos et al., 2012; Mohanty et al., 2005), which might have influenced the results. Another possible explanation could be that only certain schizophrenia-related genes are underlying the abnormal brain activation patterns during emotion processing. For example, it has been shown that COMT (catechol-O-methyltransferase) influences activation differences between siblings and controls during emotion processing. When comparing Met carriers, siblings showed higher frontal activation than controls. However, when comparing val/val carriers prefrontal activation was lower in siblings compared to controls (Lo Bianco et al., 2013). Further research on schizotypy*group and gene*group interactions on brain activation are needed to further examine these hypotheses.

The emotion regulation task resulted in brain activation patterns consistent with previous research (Buhle et al., 2013; Diekhof et al., 2011). Reappraisal led to higher activation in the bilateral VLPFC, the left DLPFC, the left DMPFC and the bilateral middle temporal gyrus. This activation pattern is in accordance with previous studies [for meta-analyses see (Buhle et al., 2013; Diekhof et al., 2011)]. Suppression of negative emotions resulted in higher activation in regions such as the bilateral insula, supplementary motor area and supramarginal gyrus which in agreement with the suppression literature (Hayes et al., 2010; Vanderhasselt et al., 2012; Vrticka et al., 2011). Furthermore, application of both strategies resulted in lower negative affect indicating successful application of the strategies. No brain activation differences were found during reappraisal between siblings and controls. This finding is in contrast with a previous study from our group which showed that although siblings were able to down-regulate negative affect through reappraisal, activation during reappraisal was lower in the superior temporal gyrus, inferior frontal gyrus and parahippocampal gyrus (van der Meer et al., 2014). The discrepancies between these findings and the current study are difficult to explain as the exact same task was applied and the sample partially overlaps. However, in the study of van der Meer et al. (2014) a low uncorrected threshold was applied due to the relatively small sample size. Furthermore, it is possible that the siblings in the study of van der Meer et al. (2014) had overall higher levels of schizotypal symptoms or genetic loading compared to the current sample, which might have put them at higher risk for psychosis, displaying more regulation deficits. In accordance with the study of van der Meer et al. (2014), the current results indicated that siblings are capable of applying reappraisal. Furthermore, siblings did not differ on the self-reported use of reappraisal, which further strengthens the idea of intact reappraisal in siblings. One other study examined brain activation patterns during reappraisal in subjects at high risk for developing psychosis, due to high schizotypy scores (Modinos et al., 2010b). The results revealed that schizotypy was related to higher prefrontal activation during reappraisal (Modinos et al., 2010b). In the current study, the siblings did not differ on schizotypy scores from the healthy controls. Therefore, it may be possible that solely an increased genetic risk for schizophrenia does not affect the ability to reappraise negative emotions. However, when schizotypy scores increase, individuals might need compensatory brain activation to apply reappraisal as indicated by Modinos et al. (2010). Besides normal activation patterns during reappraisal, the current study revealed that siblings did not differ on brain activation during suppression. Furthermore, siblings did not report more extensive use of this less efficient emotion regulation strategy, while this is often reported in patients with schizophrenia (Kimhy et al., 2012; van der Meer et al., 2009).

Several limitations of the current study need to be addressed. First, although the results revealed higher activation during reappraisal in the regions previously associated with reappraisal, we were unable to detect decreased amygdala activation during reappraisal.

The lack of amygdala deactivation in this study and previous reports (McRae et al., 2012; Opitz et al., 2012; Schulze et al., 2011) is suggested to be due to the application of a late cueing method (Ochsner et al., 2012). With this method, subjects receive the instruction to reappraise after stimulus presentation instead of before or simultaneously with stimulus presentation. The reason for applying this method was to allow participants to have a naturalistic emotional response to the negative pictures before regulation was applied (Ochsner et al., 2012). However, this late cueing method might have caused the amygdala to already habituate before reappraisal starts (Ochsner et al., 2012). Therefore, we suggest future studies to examine the frontal-limbic coupling in siblings with an early cueing paradigm. Second, the present results only represent emotion regulation in a lab-setting. The relationship of such measures to emotion regulation in daily life needs to be established in more detail, which will enhance ecological validity. Momentary assessment studies could give more insight on emotion regulation on a day-to-day basis.

|CONCLUSION

The current findings indicate that solely being a relative of a schizophrenia patient might not affect the ability to use reappraisal and suppression, at least when subjects are explicitly instructed and cued to do so. Furthermore, the neural correlates of emotion processing and emotion regulation appear to be intact in these individuals, at least under certain conditions. Future research examining interactions between group and schizotypy or specific genes might provide further information on specific risk groups in which emotion dysregulation might be presented.

|ACKNOWLEDGEMENTS

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|SUPPLEMENTARY MATERIAL

|Table S7.1 Main effects of negative emotion processing, reappraisal and suppression on BOLD response in controls and siblings.

			MNI coordinates		
	K voxels	Z	x	y	z
View negative>View neutral					
HC only					
L Middle temporal gyrus	1988	Inf	-48	-68	8
		6.58	-38	-66	18
		6.09	-46	-66	26
R Middle temporal gyrus	1822	7.65	54	-62	4
		7.46	46	-66	2
		6.08	42	-62	16
L/R Precuneus	1806	7.48	-6	-52	22
		5.25	6	-52	30
		3.47	18	-64	32
R Calcarine sulcus / Lingual gyrus	679	7.31	12	-78	8
		5.94	12	-72	-8
		4.34	20	-64	-8
L Inferior frontal gyrus, operculum	3759	6.23	-50	8	18
		5.42	-40	30	2
		5.31	-30	16	-16
R Inferior frontal gyrus, triangularis	692	5.88	54	32	4
		5.05	48	38	6
		4.60	40	12	30
L Supramarginal gyrus	364	5.58	-60	-32	36
		5.42	-62	-26	30
		4.25	-50	-28	42
L Calcarine sulcus	222	5.51	-10	-84	4
		3.76	-16	-72	8
L/R Medial superior frontal gyrus	1048	5.28	-6	54	18
		5.18	-6	50	28
		4.87	8	58	20
L Inferior parietal gyrus	144	4.47	-30	-46	52
L/R Middle cingulate gyrus	150	4.43	-2	-16	36
R Supramarginal gyrus	221	4.26	64	-32	30

Table S7.1 Continued

	K voxels	Z	MNI coordinates		
			x	y	z
		3.66	-4	-4	34
		4.18	64	-18	32
		3.55	62	-22	40
Siblings only					
R Middle temporal gyrus	2715	7.20	52	-62	2
		7.05	46	-66	6
		6.28	52	-58	16
L Middle temporal gyrus	1987	7.17	-42	-64	2
		6.84	-42	-64	12
		6.57	-54	-60	6
L/R Precuneus	1021	6.40	-6	-48	28
		4.78	-8	-62	30
		4.61	-6	-50	16
L Brainstem	494	6.01	-2	-24	-2
		5.07	0	-30	-22
		4.54	-10	-24	-10
L Inferior frontal gyrus, triangularis	553	5.09	-42	28	2
		4.53	-28	24	6
		4.48	-30	20	-10
L Superior occipital gyrus	112	4.85	-18	-82	34
		4.31	-22	-76	30
L Calcarine sulcus	316	4.82	-12	-82	2
		3.59	-10	-78	16
		3.58	-4	-84	24
R Inferior frontal gyrus, triangularis	112	4.04	52	28	6
		3.31	52	24	16
L/R Medial superior frontal gyrus	124	3.93	4	52	32
		3.70	-16	52	30
		3.61	-2	48	42
Attend negative > Attend neutral					
HC only					

|Table S7.1 Continued

			MNI coordinates		
	K voxels	**Z**	**x**	**y**	**z**
R Inferior parietal gyrus	1077	6.01	46	-48	50
		5.30	40	-60	48
		5.08	48	-64	36
R Cerebellum	896	5.75	32	-66	-32
		5.31	36	-74	-20
		4.85	22	-76	-26
L Inferior parietal gyrus	1792	5.75	-42	-52	50
		5.56	-46	-48	42
		5.47	-38	-56	44
R Inferior frontal gyrus	755	5.41	48	28	32
		5.39	34	4	56
		4.86	34	20	48
L Cerebellum	692	5.25	-42	-56	-24
		4.64	-36	-66	-26
		4.60	-40	-68	-18
L Middle frontal gyrus	350	4.86	-42	26	34
		4.60	-52	20	34
		4.35	-56	16	28
L/R Precuneus	1020	4.77	6	-60	46
		4.73	-10	-64	56
		4.52	-4	-58	60
R Inferior temporal gyrus	137	4.65	44	-66	-4
		3.45	34	-72	0
L/R Supplementary motor area	448	4.54	2	18	50
		4.37	-2	22	44
		4.00	-4	30	34
L Precentral gyrus	278	4.45	-34	-2	60
		4.25	-26	-4	58
		3.92	-22	-12	58
Siblings only					
L/R Precuneus	933	6.44	4	-66	44
		5.14	-4	-58	60
		4.81	6	-62	56

Table S7.1 Continued

	K voxels	Z	MNI coordinates		
			x	y	z
R Parietal cortex	786	5.66	44	-52	48
		5.23	48	-42	50
		5.00	46	-62	40
L/R White matter	286	5.42	-2	-22	28
		4.78	2	-36	24
		4.04	2	-12	32
L Inferior parietal gyrus	1125	5.30	-36	-56	46
		5.00	-50	-38	50
		4.72	-44	-52	52
L Inferior frontal gyrus, operculum	178	7.80	-36	-56	46
		4.25	-50	-38	50
		3.61	-44	-52	52
L Middle frontal gyrus	191	4.59	-30	56	10
		3.71	-42	48	10
		3.55	-42	42	24
R Inferior frontal gyrus, operculum	250	4.47	48	18	40
		4.19	46	32	34
		4.03	48	26	28
Reappraise > Attend negative					
HC only					
L Dorsolateral, ventrolateral and dorsomedial prefrontal cortex	7154	Inf	-48	24	-8
		7.36	-8	6	62
		6.94	-8	18	54
L Middle temporal gyrus/Angular gyrus	2752	7.07	-58	-36	-2
		7.07	-48	-60	38
		6.91	-42	-58	28
R Inferior frontal gyrus	1241	6.39	50	34	-12
		6.23	54	24	2
		5.74	48	24	-10
R Angular gyrus	559	5.70	58	-48	36
		5.68	50	-56	26
R Superior temporal gyrus	687	5.65	44	-30	-4

|Table S7.1 Continued

			MNI coordinates		
	K voxels	**Z**	**x**	**y**	**z**
		4.86	58	-2	-16
		4.85	50	8	-28
R Caudate	362	5.14	14	16	8
		4.52	8	6	2
		4.28	12	16	0
L Caudate	663	4.84	-4	-2	8
		4.51	-14	10	0
		4.51	-12	8	10
L Posterior cingulate cortex	390	4.67	-4	-42	30
		4.18	-4	-54	32
L Cerebellum	122	4.63	34	-56	-34
		4.00	34	-62	-26
L Middle cingulate gyrus	153	4.00	-4	-10	34
		3.84	0	-18	38
		3.61	-2	-18	28
Siblings only					
L Dorsolateral, ventrolateral and dorsomedial prefrontal cortex	9228	Inf	-38	18	-8
		7.67	-50	24	-6
		7.09	-52	24	4
L Middle temporal gyrus/Angular gyrus	2254	7.09	-48	-60	34
		6.74	-48	-58	24
		6.38	-50	-32	-2
L Posterior cingulate gyrus	1109	6.08	-4	-46	22
		4.43	-4	-50	34
		4.32	-12	-54	4
R Inferior frontal gyrus	1201	6.00	40	20	-10
		5.95	52	24	-4
		5.55	46	16	0
R Middle temporal gyrus	556	5.44	48	-36	-2
		4.70	48	-26	-6
		4.58	56	-10	-12
R Temporal pole	103	5.06	52	8	-22

Table S7.1 Continued

			MNI coordinates		
	K voxels	**Z**	**x**	**y**	**z**
		3.72	52	0	-34
R Angular gyrus	791	5.05	48	-50	30
		4.61	58	-48	36
		4.13	50	-52	46
R Cerebellum	127	4.38	28	-78	-26
		4.38	16	-76	-26
		3.36	6	-76	-22
Suppress > Attend negative					
HC only					
L Insula	889	6.66	-46	12	-6
		4.53	-32	14	-10
		4.08	-52	6	6
R Insula	1009	6.00	56	10	2
		5.44	46	14	-4
		4.94	36	16	6
R Supramarginal gyrus	583	5.93	60	-44	36
		5.07	62	-32	34
		4.05	62	-22	26
L/R Supplementary motor area	1201	5.62	6	2	62
		5.57	0	6	56
		5.12	-6	12	40
L Supramarginal gyrus	167	5.40	-62	-42	26
		3.69	-60	-36	38
R Middle frontal gyrus	147	5.19	28	48	26
Siblings only					
L Insula	1157	5.57	-40	14	-2
		5.03	-50	6	4
		5.03	-36	6	4
R Insula	1081	5.50	50	14	-6
		5.03	44	4	2
		4.95	42	20	-2
R Supplementary motor area	375	5.42	10	4	62

|Table S7.1 Continued

			MNI coordinates		
	K voxels	**Z**	**x**	**y**	**z**
		5.28	18	18	58
		5.05	4	10	58
R Supramarginal gyrus	764	5.13	54	-42	28
		4.78	62	-30	34
		4.51	60	-44	36
L Supramarginal gyrus	150	4.87	-58	-38	34
		3.66	-56	-42	44
R Middle frontal gyrus	136	4.32	28	42	26
L Putamen	117	4.28	-22	8	-4
Attend negative > Suppress					
HC only					
R Angular gyrus/Middle occipital gyrus	1905	6.12	34	-64	40
		4.56	44	-68	30
		4.53	26	-82	22
L Postcentral gyrus	526	5.45	-50	-10	28
		4.09	-52	-28	10
		4.06	-56	-24	2
R Middle frontal gyrus	300	5.40	36	8	54
		5.33	32	-24	48
		3.19	40	-28	42
L Middle occipital gyrus	467	5.27	-40	-72	32
		4.25	-28	-62	26
		4.24	-30	-70	22
L/R Paracentral lobule	559	5.10	4	-32	58
		3.78	-8	-32	66
		3.77	12	-50	4
L Parahippocampal gyrus	219	4.82	-22	-18	-24
		4.02	-18	-8	-20
		3.66	-28	-22	-16
R Postcentral gyrus	200	4.65	60	-6	18
		4.21	52	-6	26

Table S7.1 Continued

	K voxels	Z	MNI coordinates		
			x	y	z
Siblings only					
R Angular gyrus	222	4.43	32	-66	50
		3.72	32	-60	36
		3.27	26	-74	36
L Cuneus	175	4.22	-4	-86	18
		3.45	-2	-78	18
R Postcentral gyrus	142	3.94	12	-34	66
		3.80	4	-28	64
		3.65	6	-20	66

Abbreviations: BOLD: Blood oxygenation level dependent ; HC: Healthy controls; L: Left; R: Right

PART III
UHR INDIVIDUALS

8 Lower prefrontal activation during emotion regulation in subjects at ultra-high risk for psychosis

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|ABSTRACT

BACKGROND: Previous research has shown that patients with schizophrenia experience difficulties with emotion regulation and activate prefrontal regions to a lesser extent during reappraisal of emotional information. It has been suggested that this emotion dysregulation might precede the onset of psychosis. If so, difficulties in emotion regulation should also be present in individuals at ultra-high risk (UHR) for developing psychosis. Therefore, the aim of the current study was to examine the neural basis of reappraisal of negative pictures in subjects at UHR for developing psychosis.

METHODS: Using functional magnetic resonance imaging (fMRI), we scanned 16 UHR subjects and 16 matched healthy controls during an emotion regulation task. Furthermore, the reported use of reappraisal was examined with the emotion regulation questionnaire (ERQ).

RESULTS: Individuals at UHR for psychosis showed lower activation in the left ventrolateral prefrontal cortex during reappraisal compared to healthy controls. Furthermore, they reported less use of reappraisal in daily life.

CONCLUSION: These findings indicate that dysfunctional emotion regulation may already occur in individuals at risk for psychosis. These regulation difficulties are underpinned by lower ventrolateral prefrontal cortex activation, and may result in high negative affect, lower social functioning and high rates of psychotic symptoms in this group.

|INTRODUCTION

Although schizophrenia is widely recognized to involve impaired cognition (Reichenberg and Harvey, 2007), research is increasingly uncovering emotional abnormalities as well (Aleman and Kahn, 2005; Kring and Elis, 2013). These may pertain to deficits in perception, expression and experience of emotion. Indeed, previous research has shown that patients with schizophrenia experience high levels of negative affect (Oorschot et al., 2013). It has been suggested that this increased negative affect precedes psychotic symptoms, such as hallucinations and delusions, due to dysregulation of affect (Fowler et al., 2012; Freeman and Garety, 2003; Smeets et al., 2012). Emotion regulation can be described as the process of changing the experience and expression of emotions (Gross, 1998). Reappraisal is a frequently used emotion regulation strategy that implies a reevaluation of emotional stimuli in such a way that they become less emotionally disturbing (e.g. down-regulating negative affect) (Gross, 1998). For example, when seeing a woman cry outside of a church one could think her daughter just got married instead of thinking someone has passed away. Behavioral studies have shown that patients with schizophrenia experience difficulties with down-regulating negative affect through reappraisal (Morris et al., 2012) and tend to use less reappraisal compared to controls (Kimhy et al., 2012; Livingstone et al., 2009; van der Meer et al., 2009). Not all studies confirmed this latter finding, however (Henry et al., 2008; Perry et al., 2011).

Neuroimaging studies have shown that patients with schizophrenia have abnormal neural activation during emotion regulation (Morris et al., 2012; Strauss et al., 2013). In healthy people, activation of the dorsolateral, dorsomedial and ventrolateral prefrontal cortex has been shown to increase during reappraisal (for meta-analysis see Diekhof et al., 2011). Subsequently, amygdala activation decreases, resulting in lower negative affect (Diekhof et al., 2011). In patients with schizophrenia however, less activation has been found in the dorsolateral and ventrolateral prefrontal cortex during reappraisal compared to healthy controls (Morris et al., 2012; van der Meer et al., 2014). Furthermore, the functional connectivity between the prefrontal cortex and the amygdala has shown to be weaker in patients with schizophrenia (Morris et al., 2012).

It has been suggested that emotion regulation difficulties may precede the first onset of psychosis (Fowler et al., 2012; Smeets et al., 2012). One way of examining this hypothesis is to investigate whether emotion regulation difficulties are already present in individuals with an At Risk Mental State (ARMS), which often precedes the onset of psychosis. This ARMS is characterized by subclinical psychotic symptoms and a decline in social and global functioning (Yung and McGorry, 1996). Individuals with this ARMS have an ultra-high risk (UHR) for developing psychosis with transition rates of 29% after 2 years (Fusar-Poli et al., 2012). Remarkably, to the best of our knowledge, no studies have yet examined emotion regulation and its neural basis in this group.

Previous research has shown that UHR individuals experience difficulties during emotion processing. Specifically, compromised cognitive-emotional processing (Amminger et al., 2012a; van Rijn et al., 2011) and high levels of negative affect (Lee et al., 2008) indicate possible difficulties with emotion regulation in this group. We therefore hypothesized reduced recruitment of the emotion regulation circuitry, especially with regard to cognitive reappraisal, in individuals at UHR for developing psychosis.

|METHODS

Participants

Sixteen subjects at UHR for developing psychoses were recruited from the Mental Health Care Services in Friesland, The Netherlands. Subjects were initially screened with the Prodromal Questionnaire (PQ-16; Ising et al., 2012). A score of 6 or higher resulted in the administration of the Comprehensive Assessment of At Risk Mental States (CAARMS; Yung et al., 2005). Participants were selected if: 1) they were aged between 18 and 40 years, 2) they had a genetic risk for developing schizophrenia or CAARMS-scores in the range of At Risk Mental State (as defined in Rietdijk et al., 2010), and 3) they had an impairment in social functioning [SOFAS-scores ≤50 or a 30% drop in SOFAS scores (Goldman et al., 1992)]. This approach is in accordance with the procedure of the EDIE-NL trial (Rietdijk et al., 2010). Exclusion criteria were: 1) a history of psychosis, 2) neurological problems, and 3) MRI incompatibility. The UHR subjects were compared to 16 healthy controls without a presence or history of psychiatric or neurological disorders, matched on age, gender, education level and handedness. All participants gave written informed consent and the study was approved by the Mental Healthcare Research Ethics Committee (METIGG). All procedures were carried out according to the declaration of Helsinki. Demographic characteristics of the final sample (15 UHR and 16 controls, for the reason of exclusion see results) are presented in Table 8.1.

Behavioral measurements

Emotion Regulation Questionnaire

The emotion regulation questionnaire (ERQ; Gross and John, 2003) was applied to assess the use of the emotion regulation strategies, reappraisal and suppression. The ERQ comprises 10 items of which six examine reappraisal and four examine suppression. Participants had to rate on a 7-point scale to what extent a certain statement applied to them (strongly disagree till strongly agree). The total scores on the subscales were divided by the number of items comprising that subscale to make the subscale scores more comparable. The ERQ is a reliable and valid measure of emotion regulation (Gross and John, 2003).

Positive and Negative Affect Scale

The positive and negative affect scale (PANAS; Watson et al., 1988) was administered to examine the current affective state. The scale consists of 10 positive items (reflecting enthusiasm, activeness, and alertness) and 10 negative items (reflecting distress, anger, fear and guilt). Subjects had to rate to what extent they experienced certain mood states on a five-point scale. The PANAS has been proven a reliable and valid measure of positive and negative affect (Crawford and Henry, 2004).

Positive and Negative Syndrome Scale

To examine the clinical characteristics of the UHR individuals, the semi-structured interview Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987) was administered on the day of the functional magnetic resonance imaging (fMRI) scan. In this 30-item interview, positive, negative and general symptoms of psychosis that occurred in the week before the scan session were measured.

|Table 8.1 Mean, standard deviation and group differences for demographic data, questionnaire data, and rating scores on the emotion regulation task, and descriptives of medication status and psychotic symptoms

	HC (n=16)	ARMS (n=15)	Test statistic	
Demographics				
Gender (% male)	50%	53%	χ^2 = .02	p = .88
Age (in years)	22.1 ± 3.6	23.1 ± 4.4	t =-.70	p = .49
Education[a]	5.4 ± .6	5.2 ± .9	t = .88	p = .37
Handedness (% right)	75%	80%	χ^2 = .02	p = .88
PANAS				
Positive affect	33.4 ± 5.4	30.0 ± 7.7	t = 1.48	p = .15
Negative affect	12.4 ± 2.0	22.3 ± 6.7	t =-5.71	p < .001*
ERQ				
Reappraisal	5.1 ± 1.0	4.2 ± .9	t = 2.74	p = .01*
Suppression	4.5 ± 1.7	4.1 ± 1.2	t = .85	p = .40
Rating scores				
Attend neutral	1.1 ± .2	1.3 ± .4	U = 64.0	p = .03[b]
Attend negative	2.4 ± .5	2.5 ± .7	U = 108.5	p = .65
Reappraise	1.8 ± .4	2.1 ± .6	U = 86.5	p = .18
Current medication status (n)				
Antipsychotics	0	0		
Antidepressants (SSRI)	0	4		
Methylphenidate	0	2		
Other medication	1	1		
PANSS				
Positive symptoms	n/a	13.1 ± 2.7		
Negative symptoms	n/a	10.5 ± 2.5		
General symptoms	n/a	28.3 ± 6.1		

* significant at p<.05; [a] Education according to Verhage, 1964; [b] not significant at the corrected p-value of p<.017.
Abbreviations: ERQ: Emotion regulation questionnaire; PANAS: Positive and Negative Affect Scale; PANSS: Positive And Negative Syndrome Scale; SSRI: Selective Serotonin Reuptake Inhibitor

Emotion Regulation Task

The emotion regulation task (adapted from Ochsner and Gross, 2005) consisted of three conditions, Attend Neutral, Attend Negative, and Reappraise. The stimuli consisted of 44 negative and 22 neutral pictures from the International Affective Picture System (IAPS). Each trial was constructed as follows: First, a picture appeared with the instruction to 'view' the picture (View condition, 2s). Subsequently, the word 'view' changed in either 'reappraise' or 'attend' (Regulation condition, 4s). During reappraise, participants had to reappraise the picture in such a way that it became less emotionally disturbing. During attend, participants

were instructed to look closely at the picture and not change the way they were feeling. The neutral pictures were always paired with the 'attend' instruction. Negative pictures were paired with either reappraise (22 pictures) or attend (22 pictures). Following regulation, a black screen appeared (Linger, 2s). After that, participants were asked to rate how negative they were feeling on a four-point rating scale (1=not negative at all; 4=extremely negative) (3s). Subsequently, the word 'relax' appeared (4s), followed by a black screen (0.5s) to alert participants that the next trial was coming. One trial lasted 15.5 seconds. After 9 or 10 trials, a fixation cross appeared for 20 seconds.

To ensure correct application of the reappraisal strategy, a short training was given prior to the fMRI scan. During this training, participants practiced the reappraisal strategy by telling the researchers how they would apply the strategy in response to several negative pictures.

Image acquisition

MRI data were acquired using a 3.0 Tesla whole body scanner (Philips Intera Achieva, Best, NL), equipped with an 8-channel SENSE head coil located at the University Medical Center Groningen. The functional images were acquired by a T2-weighted echo producing 37 slices of 3.5 mm thick with no gap. The images were slightly tilted (30 degrees) to prevent artifacts from the nasal cavities. The functional scans were made in the axial plane (TR=2 s; TE=30 s; flip angle (α)=70°; FOV=224.0, 129.5, 224.0; in-plane resolution 64x62 pixels; isotropic voxels of 3.5 mm) and were scanned interleaved. The T1-weighted anatomical image (170 slices; isotropic voxels of 1 mm; TR=9 ms; TE=3.54 ms; α=8°; FOV=256 mm) was acquired in the bicommissural plane, covering the whole brain.

Statistical analyses

Behavioral analyses were performed using SPSS 20 (SPSS Inc., Chicago, IL, USA). To examine differences between the UHR group and controls on demographic variables, t-tests and Chi-square tests were performed. To examine possible differences on the PANAS and ERQ scores, two-sample t-tests were performed. The significance level for these tests was set at $p<.05$. Due to non-normality of the rating scores from the emotion regulation task, a Friedman's ANOVA was applied to examine the main effect of condition on negative affect per group ($p<.05$). Post hoc analyses were performed with a Wilcoxon signed-rank test ($p<.017$, correcting for 3 tests, applying a Bonferroni correction). To examine group differences on the rating scores of the emotion regulation task, Mann-Whitney U tests were performed ($p<.017$, correcting for 3 tests, applying a Bonferroni correction).

The fMRI analyses were performed using Statistical Parametric Mapping (SPM8) (www.fil.ion.ucl.ac.uk) running in Matlab 7 (The MathWorks Inc., Natrick, MA, USA). First, all images were checked for artifacts. Second, slice timing was applied and the functional images were spatially realigned, resliced and coregistered to the anatomical scan. The anatomical images were segmented. Furthermore, the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) approach was used to create a gray matter template based on the gray matter segmented images to enhance the accuracy of inter-subject alignment. This template was used to normalize the functional images to and affine-transform them into Montreal Neurological Institute (MNI) stereotactic space. A Gaussian kernel of 6 mm FWHM was applied to smooth the data. Subject head movement greater

than 3 mm in more than one direction resulted in exclusion of the data (one participant).

Thirteen task-related regressors were modeled with a boxcar function convolved with a hemodynamic response function. The regressors View and Relax were divided into View/Relax neutral and View/Relax negative. The other regressors (Regulation, Linger and Rating) were subdivided into a Reappraise, Attend Negative and Attend Neutral part. Additionally, the realignment parameters and the first derivatives thereof were entered as covariates to correct for possible effects related to head motion. Four contrasts were made for each participant: 1) View Neutral versus Fixation; 2) View Negative versus View Neutral; 3) Attend Negative versus Attend Neutral; 4) Reappraise versus Attend Negative.

To examine task-related activation, one sample t-tests in healthy controls and UHR subjects were conducted separately. Sex and handedness were entered as covariates of no interest. Two-sample t-tests were performed to examine group differences on task-related activation for all four abovementioned contrasts, with sex and handedness as covariates. To limit possible false positives due to multiple comparisons, effects had to meet $p<.05$ Family-Wise Error (FWE) corrected at the cluster level to be considered statistically significant (initial height-threshold for all analyses was set at $p<.001$). Because of specific hypotheses regarding the amygdala (see introduction), a Small Volume Correction (SVC) was applied for this region.

|RESULTS

Demographic and behavioral results

One subject from the UHR group was excluded from the analyses because of poor data quality due to head motion. The final sample therefore consisted of 15 UHR subjects and 16 healthy controls.

The UHR individuals and healthy controls did not differ significantly on gender, age, education and handedness (see Table 8.1 for test statistics). UHR individuals reported to use less reappraisal ($p=.01$) on the ERQ, while the reported use of suppression did not differ between the groups (see Table 8.1). Furthermore, UHR individuals reported more negative affect before scanning, but no significant differences in positive affect were found (see Table 8.1).

On the ratings during the emotion regulation task, a main effect of condition (Attend Neutral, Attend Negative, Reappraise) was found in both the controls ($\chi^2(2)=32.0$, $p<.001$) and the UHR group ($\chi^2(2)=25.7$, $p<.001$). All participants rated the negative pictures as more negative compared to the neutral pictures (controls: $Z=3.5$, $p<.001$; UHR: $Z=3.4$, $p=.001$). Furthermore, participants were capable of reducing their negative affect during reappraisal (controls: $Z=-3.5$, $p<.001$; UHR: $Z=-3.2$, $p=.001$) compared to attending negative pictures. No group differences were found on the ratings of negative affect, apart from a slightly higher rating of negative affect after attending neutral pictures in the UHR group ($p=.03$, not reaching the multiple comparison threshold).

Neuroimaging results

Main effect of the emotion regulation task

The first two seconds of viewing a negative picture, compared to neutral, revealed higher activation in the control group in the bilateral middle temporal gyrus, retrosplenial cortex, bilateral fusiform gyrus and right ventrolateral prefrontal cortex (VLPFC) (see supplementary

|Table 8.2 Summary of significant brain activation differences between UHR individuals and controls during emotion processing and reappraisal

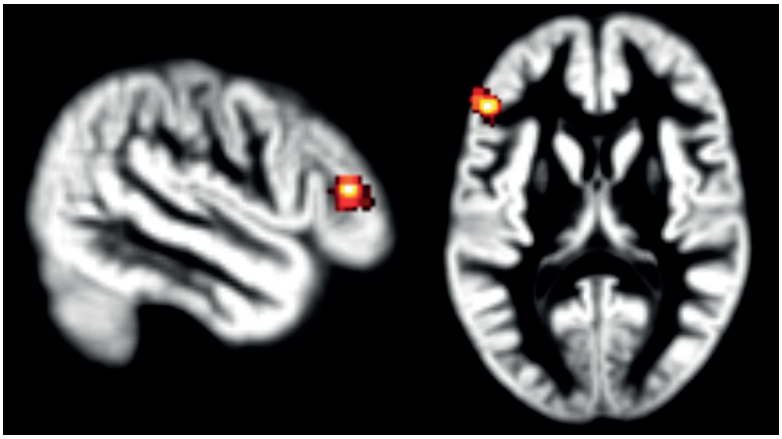
			MNI coordinates		
Brain region	**k voxels**	**Z**	**x**	**y**	**z**
View Neutral > Fixation					
Controls > UHR					
L Temporal pole	158	5.34	-36	22	-22
		4.11	-28	12	-24
L/R Posterior cingulate gyrus	273	4.23	8	-50	28
		3.99	-4	-26	22
		3.80	0	-40	18
View Negative > View Neutral					
UHR > Controls					
L/R Posterior cingulate gyrus	144	3.73	-6	-52	30
		3.67	8	-50	28
		3.64	0	-42	32
Reappraise > Attend Negative					
Controls > UHR					
L Ventrolateral prefrontal cortex	168	4.76	-46	26	12

Abbreviations: L: left; MNI: Montreal Neurological Institute; R: right; UHR: ultra-high risk

Table S8.1). Furthermore, the left [p_{svc}=.02; k=14; Z=3.8;-22,-6,-18 (x,y,z)] and right [p_{svc}=.01; k=19; Z=3.6; 26,0,-24 (x,y,z)] amygdala were higher activated during negative picture viewing compared to neutral in the control group. The UHR subjects revealed a similar pattern of activation (see supplementary Table S8.1) and higher activation in the left amygdala [p_{svc}=.01; k=36; Z=3.9;-26,2,-24 (x,y,z)] during negative picture viewing. The four seconds of attending a negative picture, after the first two seconds of viewing, revealed higher activation in the bilateral inferior occipital gyrus and left calcarine sulcus in the control group (see supplementary Table S8.1) in comparison to attending neutral pictures. The UHR subjects also activated the visual cortex, while attending negative pictures, together with the dorsomedial prefrontal cortex (DMPFC) and the orbitofrontal cortex (see supplementary Table S8.1). Reappraising negative pictures compared to attending negative pictures resulted in increased activation in the bilateral VLPFC, the left middle and superior temporal gyrus and in the DMPFC in the control group (see supplementary Table S8.1). In the UHR group, no significant activation differences were found between reappraising and attending negative pictures. No activation decreases were found in the abovementioned contrasts.

Activation differences between the UHR and control group

Viewing negative pictures, compared to viewing neutral pictures, resulted in higher activation in the posterior cingulate cortex (PCC) in the UHR group compared to the control



|Figure 8.1 Higher activation in the left VLPFC in healthy controls compared to UHR subjects for the contrast reappraisal > attend negative. Results are displayed at p<.001 with a p<.05 FWE correction at the cluster level and overlaid on a normalized gray matter template based on the segmented T1 images of all participants.

group (see Table 8.2). This result was caused by less activation in the PCC during neutral picture viewing in the UHR group (see Table 8.2). During reappraisal, compared to attend negative, less activation in the left VLPFC was observed in the UHR group compared to controls (see Figure 8.1 and Table 8.2). No other activation differences were found between the UHR and the control group.

|DISCUSSION

The aim of the current study was to examine whether individuals at ultra-high risk (UHR) for developing psychosis differ from healthy controls in brain activation during emotion regulation. The results revealed less activation of the left ventrolateral prefrontal cortex (VLPFC) in UHR individuals during reappraisal. Furthermore, the UHR group reported less use of reappraisal in daily life and showed higher rates of negative affect, compared to control subjects.

Our primary interest pertained the brain regions involved in reappraisal of emotion. Reappraising negative pictures resulted in activation of the left temporal cortex, bilateral VLPFC and bilateral dorsolateral and dorsomedial prefrontal cortex in healthy controls. This activation pattern is consistent with previous neuroimaging literature (Buhle et al., 2013; Diekhof et al., 2011). In line with our hypothesis, the UHR individuals activated the left VLPFC to a lesser extent during reappraisal compared to controls. The left VLPFC is consistently activated across reappraisal studies in healthy subjects (for meta-analyses see Buhle et al., 2013; Diekhof et al., 2011) and is specifically involved in the cognitive regulation of feelings (Ochsner et al., 2002). Lower activation in the VLPFC has previously been reported in patients with schizophrenia during reappraisal (Morris et al., 2012). The current results show that this lower activation pattern might already be present in individuals at high risk for psychosis. Notably, in healthy college students with a slightly increased risk for developing psychosis (i.e. high psychosis proneness), higher activation in the VLPFC was found during reappraisal (Modinos et al., 2010b). The authors hypothesized that this higher activation pattern might reflect a compensatory mechanism. This suggests that there might be an inverted U-shape pattern of VLPFC activation in risk groups for developing psychosis during reappraisal. Groups with a slightly increased risk might still be capable of applying reappraisal through compensatory VLPFC activation, while groups at UHR might not show this compensation

anymore, reflected by lower VLPFC activation.

Activation of the left VLPFC has been shown to be positively correlated with reappraisal success (Wager et al., 2008). This suggests that UHR individuals might be less successful in applying reappraisal, which has previously also been reported in patients with schizophrenia (Morris et al., 2012). However, the behavioral task data in the current study revealed that the UHR group was equally capable in reducing negative affect through reappraisal as the control group during the task. Nevertheless, the UHR group did report a tendency to use less reappraisal in daily life. This lower reported use of reappraisal has also been found in patients with schizophrenia (Kimhy et al., 2012; Livingstone et al., 2009; van der Meer et al., 2009), although not in all studies (Henry et al., 2008; Perry et al., 2011). One possible explanation for the non-significant group differences on negative affect after reappraisal during the task, could be that UHR individuals were still capable of applying reappraisal in a structured laboratory setting, even though they could not fully recruit the relevant circuitry. Consequently, application of reappraisal might fail in more complex daily life situations, as reflected by the lower tendency to use reappraisal in daily life. Another possibility could be that the four-point rating scale was not sensitive enough to pick up small group differences in negative affect reduction after reappraisal.

Furthermore, the results showed a higher level of negative affect in UHR individuals prior to the fMRI scan, which is typical for this group (Lee et al., 2008). Lower reported use of reappraisal has been associated with higher levels of negative affect (Andreotti et al., 2013; Perry et al., 2011). This might indicate that difficulties with emotion regulation in the UHR group result in more negative affect. More negative affect is suggested to precede psychotic episodes, presumably due to difficulties with emotion regulation (Fowler et al., 2012; Smeets et al., 2012). Our results support this hypothesis by revealing emotion regulation difficulties and more negative affect in the UHR group. Notably, previous research has revealed associations between reappraisal difficulties and both lower social functioning (Gross, 2002) and higher levels of psychotic symptoms (Perry et al., 2011). This suggests that emotion regulation difficulties, reflected by less VLPFC activation, might indeed put individuals at increased risk for developing psychosis.

Viewing negative pictures, compared to neutral, revealed higher activation in various emotion processing areas, such as the amygdala, middle temporal gyrus and fusiform gyrus (Adolphs, 2002a), in both control and UHR subjects. No differences between groups were found during processing of negative emotions, except for higher posterior cingulate (PCC) activation in the UHR group. This result was caused by lower PCC activation in the UHR group during neutral picture viewing. The only other fMRI-study examining the neural basis of emotion processing in people at UHR for schizophrenia found activation differences only when examining interaction effects with age (Gee et al., 2012). Furthermore, neuroimaging studies on emotion processing in relatives of patients, and therefore with a slightly elevated risk for developing schizophrenia, have produced equivocal results. Some reported increased (Li et al., 2012; van Buuren et al., 2011) or decreased activation (Lo Bianco et al., 2013; Venkatasubramanian et al., 2010), while others were unable to find activation differences during negative emotion processing (Barbour et al., 2012). Therefore, further research is necessary to investigate the neural basis in individuals at (ultra-high) risk for schizophrenia.

Several limitations of this study should be addressed. First, although we replicated the robust finding that reappraisal is associated with increased prefrontal activation, we did not observe a subsequent decrease of amygdala activation (Diekhof et al., 2011). A number of other studies also failed to find such a decrease in amygdala activation (McRae et al., 2012; Opitz et al., 2012; Schulze et al., 2011). This has been attributed to the late cueing method,

that we also applied (i.e. giving the instruction to reappraise after 2s of stimuli presentation, and not immediately). This late cueing method was applied to allow participants to have a naturalistic emotional response to the negative valence picture before regulation starts (Ochsner et al., 2012). However, this late cuing method might have caused the amygdala to respond and habituate already before reappraisal starts (Ochsner et al., 2012). Therefore, future studies should investigate the frontal-limbic coupling in UHR subjects during reappraisal with an early cueing paradigm. Second, due to our small group sizes we were unable to compare UHR individuals who developed a psychotic episode with UHR individuals who did not. We recommend future research to examine whether our findings can be replicated in larger groups and to examine whether the lower VLPFC activation during reappraisal is specific to subjects who later on develop a psychosis or whether the lower activation is present in the entire UHR group.

|CONCLUSION

These results may indicate that emotion regulation difficulties, and the associated reduction in activation of the VLPFC, already occur in individuals at UHR for developing psychosis. These regulation difficulties could help explain the higher negative affect and lower social functioning that might put these individuals at increased risk of developing schizophrenia. Future studies should therefore explore whether the UHR group might benefit from emotion regulation training.

|ACKNOWLEDGEMENTS

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|SUPPLEMENTARY MATERIAL

|Table S8.1 Main effects of negative emotion processing and reappraisal on BOLD responses

			MNI coordinates		
	k voxels	**Z**	**x**	**y**	**z**
View negative > View neutral					
HC only					
R Middle temporal gyrus	1434	6.15	44	-60	8
		5.77	52	-66	4
		5.77	48	-72	-2
L Middle temporal gyrus	1261	5.76	-44	-64	6
		5.65	-48	-72	8
		5.43	-48	-76	0
L/R Retrosplenial cortex	572	5.52	-6	-56	12
		4.98	8	-52	8
		3.55	18	-56	18
L Fusiform gyrus	114	5.48	-26	-36	-18
R Ventrolateral prefrontal cortex	441	5.45	46	10	28
		4.47	32	8	30
		3.66	48	22	24
R Fusiform gyrus	161	4.85	42	-46	-20
		3.23	38	-64	-18
L/R Brainstem	139	4.50	-2	-28	-6
		4.01	10	-26	-18
L/R Brainstem	165	4.28	-4	-18	-18
		4.00	8	-16	-14
		3.98	6	-8	-8
R Middle temporal gyrus	147	4.13	54	-6	-18
		3.51	48	-12	-14
		3.48	50	2	-26
UHR only					
R Middle temporal gyrus	1813	6.25	44	-58	8
		6.24	54	-68	4
		4.99	46	-74	-2
L Middle temporal gyrus	1715	5.59	-48	-72	8
		5.52	-40	-60	12

|Table S8.1 Continued

			MNI coordinates		
	k voxels	**Z**	**x**	**y**	**z**
		4.77	-38	-62	26
R/L Thalamus	995	5.17	8	-26	-8
		4.98	-4	0	8
		4.60	0	24	0
L Ventrolateral prefrontal cortex	192	5.17	-42	24	-16
		4.91	-46	28	4
		4.60	-42	18	-26
R Fusiform gyrus	129	5.15	40	-50	-20
L/R Retrosplenial cortex	1684	5.12	-6	-56	26
		5.04	-2	-40	34
		4.95	8	-52	28
L/R Dorsomedial prefrontal cortex	699	4.80	-4	60	30
		4.18	-2	50	38
		4.04	6	50	32
R Ventrolateral prefrontal cortex	187	4.38	50	18	22
		3.47	42	24	22
		3.61	48	22	12
L Middle temporal gyrus	160	4.33	-58	-2	-18
		3.91	-46	6	-30
		3.85	-50	-10	-22
L/R Cerebellum	157	4.04	4	-58	-48
		3.79	14	-52	-44
		3.78	-10	-54	-46
Attend negative > Attend neutral					
HC only					
R Inferior occipital gyrus	2533	6.74	40	-74	-2
		6.59	48	-74	-8
		5.04	24	-92	4
L Inferior occipital gyrus	1339	5.72	-44	-78	-12
		5.39	-48	-74	2
		5.31	-38	-82	-2
L Calcarine sulcus	131	4.14	-6	-92	4
		4.28	-14	-90	-2

|Table S8.1 Continued

			MNI coordinates		
	k voxels	**Z**	**x**	**y**	**z**
UHR only					
R Inferior occipital gyrus	2564	5.86	48	-74	-8
		5.47	42	-74	-2
		5.09	32	-90	-8
L Inferior occipital gyrus	1645	5.10	-48	-74	2
		4.71	-36	-84	-16
		4.63	-42	-78	-12
L/R Precuneus	474	4.85	0	-56	52
		4.07	8	-66	50
		3.96	-2	-64	50
R Cerebellum	240	4.56	8	-82	-30
		3.78	6	-82	-20
		3.73	4	-78	-40
L/R Dorsomedial prefrontal cortex	114	4.15	2	26	40
		3.99	-6	14	42
L Orbitofrontal cortex	223	3.80	-42	50	-2
		3.77	-40	56	6
Reappraise > Attend negative					
HC only					
L Inferior frontal gyrus triangular part	1397	5.49	-50	24	8
		5.38	-44	30	-8
		4.79	-52	20	-2
L Middle temporal gyrus	195	5.25	-50	4	-28
		5.17	-46	4	-38
		3.60	-52	8	-16
L/R Dorsomedial prefrontal cortex	1190	4.74	-4	36	34
		4.64	-14	14	50
		4.49	12	24	52
L Middle temporal gyrus	129	4.48	-62	-38	-6
		3.51	-54	-32	-10
L Superior temporal gyrus	471	4.40	-56	-46	20
		4.21	-44	-60	22
		4.19	-52	-60	34

|Table S8.1 Continued

			MNI coordinates		
	k voxels	**Z**	**x**	**y**	**z**
R Inferior frontal gyrus operculum	170	3.77	60	18	8
		3.76	46	20	-2
		3.32	52	32	-8
UHR only					
No significant results					

Abbreviations: BOLD: blood oxygenation level dependent; HC: healthy controls; L: Left; MNI: Montreal Neurological Institute; R: Right; UHR: Ultra-high risk

9 Summary and general discuson

Patients with schizophrenia have difficulties in the processing and regulation of emotions (Kring and Elis, 2013; van der Meer et al., 2009). Furthermore, aberrant brain activation patterns during emotion processing and regulation have been reported in these patients (Morris et al., 2012; Taylor et al., 2012; van der Meer et al., 2014). The aim of this thesis was to examine whether these difficulties and aberrant neural correlates are already present in subjects at high risk for developing psychosis. To this extent, we examined brain activation during emotion processing and regulation in three groups at (putative) high risk for psychosis, namely individuals with high scores on alexithymia, individuals at genetic risk for psychosis (siblings of patients) and individuals at ultra-high risk (UHR) for psychosis. Moreover, we examined whether regions involved in emotion processing and regulation showed structural abnormalities in these increased risk groups. Examining emotion dysregulation in groups at high risk for psychosis may provide insight into emotion dysregulation as a possible vulnerability factor for developing psychosis, and subsequently, may give indications for therapies in these at-risk groups. In this chapter, the main findings of the presented studies (**chapter 2-8**) will be summarized briefly first. Subsequently, the findings will be discussed and recommendations for future research and clinical implications will be highlighted.

|SUMMARY

To integrate previous neuroimaging literature on emotion processing in alexithymia, we performed a meta-analysis, presented in **chapter 2**. The results showed that alexithymia is associated with higher activation in the dorsal anterior cingulate cortex (ACC) during the processing of negative and positive stimuli. This may indicate stronger recruitment of neural resources, possibly due to a higher cognitive demand in individuals with high scores on alexithymia. During negative emotion processing, alexithymia was related to lower activation in 1) an emotional attention network (e.g. amygdala and visual cortex), 2) brain areas with mirror neuron properties (e.g. the dorsal premotor cortex, the parietal cortex and the supplementary motor area) and 3) the dorsomedial prefrontal cortex (DMPFC). This lower activation was suggested to underlie decreased attention to negative stimuli, poor empathic skills and emotion regulation problems related to alexithymia. Furthermore, insula and precuneus activation was lower during positive emotion processing, probably underlying lower positive affect. Taken together, these results support the hypothesis that alexithymia is associated with aberrant brain activation patterns during emotion processing, which may underlie the emotion processing difficulties that individuals with high alexithymia experience.

In **chapter 3**, we examined whether the two alexithymia dimensions (i.e. cognitive and affective alexithymia) are related to different morphological profiles. We found that in non-clinical individuals the cognitive alexithymia dimension was related to lower gray matter volume (GMV) in the dorsal ACC, a region suggested to be involved in emotion recognition and regulation. In contrast, the affective dimension appeared to be related to lower GMV in the medial orbitofrontal cortex (MOFC), which may play a role in emotional arousal and imagination. Furthermore, the affective dimension was associated with lower white matter volume in the superior longitudinal fasciculus which may be involved in fantasizing and imagination. These findings support the idea of two separable alexithymia dimensions as they appear to be subserved by dissociable structural correlates.

In **chapter 4**, we examined the hypothesis of alexithymia as an emotion regulation deficit using fMRI. The results revealed that, in a group of non-clinicial individuals, alexithymia was associated with lower activation in emotion attention and recognition areas during emotion perception. However, brain activation did not differ as a function of alexithymia during

emotion regulation (i.e. suppression and reappraisal). Furthermore, individuals with high scores on alexithymia were equally capable of applying emotion regulation as individuals with low scores. These results suggest that alexithymia may arise from an early emotion perception deficit instead of compromised neural circuits subserving explicit emotion regulation.

In **chapter 5**, we examined whether alexithymia is associated with the degree of risk for psychosis. The results revealed that both patients with schizophrenia as well as individuals at high risk for psychosis had higher levels of cognitive alexithymia combined with low or normal levels of affective alexithymia compared to controls. Furthermore, subjects at UHR for psychosis had higher cognitive alexithymia scores compared to siblings of patients with schizophrenia. This suggests that cognitive alexithymia may be part of the vulnerability for psychotic disorders.

The aim of **chapter 6** was to examine whether siblings differ from controls on gray matter volume and concentration. The results revealed no differences between these two groups on both gray matter measures. Furthermore, specifically selecting subjects on age, genetic loading or schizotypy did not alter these findings. Thus, gray matter as measured through voxel-based morphometry, might not be a suitable endophenotype for schizophrenia.

To examine the use and neural correlates of emotion regulation in subjects at genetic risk for psychosis, we performed an fMRI-study on emotion regulation in siblings of patients with schizophrenia (**chapter 7**). No differences were found between the sibling group and the control group on the use of emotion regulation, nor the underlying neural correlates. These non-significant findings suggest that solely being a sibling of a patient with schizophrenia by itself may not imply impaired emotion regulation capacities.

In **chapter 8**, we studied emotion regulation in subjects at UHR for developing psychosis. The results revealed lower ventrolateral prefrontal cortex (VLPFC) activation during reappraisal in the UHR group as compared to the control group. The VLPFC is involved in the cognitive regulation of emotions and VLPFC activation has been positively related to reappraisal success. Furthermore, UHR individuals reported less use of reappraisal in daily life compared to controls. These results support the hypothesis that emotion dysregulation may already be present before the onset of a psychotic disorder.

|ALEXITHYMIA

The neural correlates of emotion processing in alexithymia

Alexithymia is a putative risk factor for developing psychosis (van 't Wout et al., 2007; van der Meer et al., 2009) and emotion processing deficits lie at its core (Grynberg et al., 2012; Nemiah and Sifneos, 1970; Taylor et al., 1997). Therefore, in this thesis, we examined alexithymia-related neural correlates of emotion processing in order to gain more knowledge on the underlying neural basis of these deficits. As outlined in **chapter 1**, emotion processing is not a unitary construct, but instead consists of different phases (Smith and Kirby, 2000). The results of this thesis show that alexithymia is associated with aberrant structural and functional neural correlates involved in the early phases of emotion processing (e.g. appraisal detection and emotion generation), while the neural correlates of emotion regulation, a later phase, may remain intact.

Appraisal detection

Appraisal detection is the phase in which a relevant stimulus is detected and attention is drawn toward this stimulus (Smith and Kirby, 2000). The findings in this thesis show that alexithymia is associated with lower activation in regions involved in early appraisal detection, namely the amygdala and visual cortex (**chapter 2** and **4**). More specifically, these regions are responsible for early emotion detection and directing (visual) attention toward emotional stimuli (Adolphs, 2002a; Vuilleumier, 2005). The findings of abnormal activation during early appraisal detection in alexithymia are supported by two EEG studies, which showed aberrant event-related potentials (ERP) in alexithymia already during the early phases of emotion processing (Delle-Vigne et al., 2014; Goerlich et al., 2012). This aberrant activation may be specifically underlying the emotional attention deficits (Mueller et al., 2006; Suslow et al., 2003) and the emotion identification difficulties (Lane et al., 2000) in alexithymia. Recent research has supported this hypothesis by showing lower amygdala activation in high versus low alexithymia during an emotional facial identification task (Jongen et al., 2014). This lower activation was associated with worse task performance (Jongen et al., 2014). Furthermore, our findings and previous reports specifically point to an association between lower amygdala activation and the difficulties in identifying feelings in alexithymia (**chapter 4**; Jongen et al., 2014; Kugel et al., 2008; Pouga et al., 2010; Reker et al., 2010). Therefore, we suggest that alexithymia is related to lower activation in early appraisal detection regions, which might be specifically underlying the difficulties in identifying feelings.

Emotional stimuli may trigger associated knowledge, such as memories, which can influence appraisal detection (Smith and Kirby, 2000). For example, when seeing a dog, the memory of being bitten by a dog last month can result in a stronger appraisal detection (e.g. higher amygdala activation). It has been suggested that the (para)hippocampal formation is involved in creating this association between memories and perceived emotional stimuli (Adolphs, 2002a; Smith and Kirby, 2000). The results of **chapter 4** revealed lower activation in the parahippocampus in association with alexithymia during negative emotion processing. This is in accordance with recent published data (Jongen et al., 2014). Furthermore, another recent study has shown that high levels of alexithymia combined with early life stress resulted in lower hippocampal volume (Aust et al., 2014). Even though further research on the relation between alexithymia and the (para)hippocampal complex is necessary, these findings suggest that alexithymia might be related to impaired emotional memory associations. This, in turn, can result in lower appraisal detection as the appraisal detection might be less influenced by associated knowledge.

Emotional response and emotional awareness

After appraisal detection, an emotional response is formed and, if strong enough, emotional awareness is generated (Smith and Kirby, 2000). The MOFC is a region involved in the formation of this emotional response by generating an affective state (Rothkirch et al., 2012; Rudrauf et al., 2009). The findings of **chapter 3** showed that alexithymia was associated with lower GMV in this region, which was specifically related to higher scores on the affective alexithymia dimension (i.e. lower levels of emotional arousal). This suggests that in individuals with high affective alexithymia, the capacity to generate an affective state might be lower.

Furthermore, **chapter 3** showed lower GMV in the dorsal ACC in relation to alexithymia. This region has been associated with the cognitive processing of emotions and is thought to be involved in processes such as emotion recognition and emotional awareness (Etkin, 2010;

Etkin et al., 2011). This lower dorsal ACC volume may indicate a lower functional capacity of this region, which could support the proposed inverted U-shape hypothesis from **chapter 2**. This inverted U-shape hypothesis suggests that during simple emotion processing tasks, such as passive viewing of emotional pictures, activation of the dorsal ACC is higher in alexithymia compared to controls, which may reflect a compensatory mechanism. When task difficulty increases, for example when identification of emotional stimuli is required, this activation may drop and hence, task performance may decline. This hypothesis was proposed because the meta-analysis in **chapter 2** revealed higher dorsal ACC activation in alexithymia, while several other studies had reported lower activation in this region (Kano et al., 2003; Karlsson et al., 2008; Moriguchi et al., 2007). Furthermore, such an inverted U-shape of ACC activation has previously been reported in patients with obsessive compulsive disorder (Koch et al., 2012).

Recently, two new studies on the neural correlates of alexithymia reported lower ACC activation in individuals with high alexithymia (Chester et al., 2014; Jongen et al., 2014). The first study provided support for the inverted U-shape hypothesis as lower dorsal ACC activation was found during a more cognitive demanding emotion recognition task (Jongen et al., 2014). Furthermore, this task was more difficult for individuals with alexithymia than controls as reflected by their lower performance (Jongen et al., 2014). The second study reported lower dorsal ACC activation in alexithymia during a social exclusion task (Chester et al., 2014). This result might be explained by a second hypothesis regarding the diverse findings on dorsal ACC activation in alexithymia, which was originally proposed by Kano and Fukudo (2013) and further described in **chapter 2**. This hypothesis suggests that stimuli with a physical context, such as pain, might elicit higher dorsal ACC activation in alexithymia. This hypothesis was based on several studies showing higher ACC activation in alexithymia during pain-related tasks (Kano et al., 2007; Moriguchi et al., 2007). Based on indications that the neural correlates of social exclusion and pain are quite similar (Kross et al., 2011), one would expect higher dorsal ACC activation in alexithymia during social exclusion. However, Chester et al. (2014) reported lower ACC activation related to alexithymia. This discrepancy might be explained by the suggestion that social exclusion during a cyberball task (as applied by Chester et al., 2014) is less intense than physical pain or social exclusion in daily life and the emotional content might therefore be more difficult to detect (Kross et al., 2011). This could indicate that individuals with alexithymia might have experienced more difficulties in identifying social exclusion in the study of Chester et al. (2014), which could explain the lower dorsal ACC activation. If dorsal ACC activation dropped due to higher task difficulty, this would be in line with our inverted U-shape hypothesis of dorsal ACC activation in alexithymia. However, this hypothesis regarding the findings of Chester et al. (2014) remains speculative as no behavioral measures on the level of experienced rejection were included during this task.

Taken together, recent literature provides further support for the inverted U-shape hypothesis of dorsal ACC activation in alexithymia, which might be caused by lower capacity of this region as reflected by the lower dorsal ACC volume. This suggests that when emotional responses are strong enough to reach awareness, individuals with alexithymia might experience difficulties recognizing and analyzing these feelings due to aberrant functioning of the dorsal ACC.

Emotion regulation

After the generation of a subjective emotional state, emotion regulation may take place to change this emotional response (Gross, 1998). Alexithymia has long been regarded an

emotion regulation deficit (Aleman, 2005; Taylor et al., 1997; Taylor and Bagby, 2004). Therefore, we suggested in **chapter 2** that the lower DMPFC activation found in association with alexithymia during emotion processing might have been underlying emotion regulation difficulties. However, in **chapter 4**, we examined the neural correlates of emotion regulation and did not find any activation differences related to alexithymia. Furthermore, individuals with high levels of alexithymia were equally capable of down regulating their negative affect through emotion regulation as individuals with low alexithymia levels. It is possible that the lower DMPFC activation found in **chapter 2** did not reflect less efficient emotion regulation as this is not the only function of this region. The DMPFC is also involved in the generation of the emotional response and subsequently, the formation of an affective state (Kober et al., 2008; Phillips et al., 2003). Therefore, the lower DMPFC activation during emotion processing reported in our meta-analysis (**chapter 2**), may also indicate abnormalities during the generation of an affective state. Another possibility is that the lower DMPFC activation reflected difficulties with implicit emotion regulation, instead of explicit emotion regulation, since implicit regulation often takes place when people are presented with negative stimuli (Gyuruk et al., 2011). In our emotion regulation task (**chapter 4**), participants were explicitly trained and cued to perform emotion regulation, which might have made it less difficult to regulate negative affect. Furthermore, we should acknowledge the possibility that our emotion regulation task may not have been difficult enough to find subtle emotion regulation deficits. For example, we used static photographs which are probably more easy to regulate than real life situations (for more discussion on the emotion regulation task, see page 169). Future research should use more realistic stimuli, such as movie clips, to further examine emotion regulation in alexithymia. However, our results do show that when explicitly trained and cued to perform reappraisal in a lab-based setting, the neural correlates of emotion regulation appear intact in alexithymia.

In conclusion, alexithymia seems to be related to functional and structural correlates in regions involved in appraisal detection, the generation of emotional responses and the awareness thereof. This pattern resembles the pattern of lower activation in patients with schizophrenia during emotion processing (Taylor et al., 2012; Li et al., 2010). However, while in patients lower prefrontal activation during emotion regulation has been found (van der Meer et al., 2014; Morris et al., 2012), the neural correlates of emotion regulation appear to be intact in alexithymia. At least, when individuals with alexithymia are explicitly trained and cued to perform emotion regulation. This suggests that alexithymia might be more an emotion perception and generation deficit than an emotion regulation disorder.

The two dimensions of alexithymia

As described in **chapter 1**, it has been suggested that alexithymia might not be a uniform construct, but may comprise of two different dimensions, a cognitive and affective alexithymia dimension (Vorst and Bermond, 2001). The cognitive dimension consists of the identifying, analyzing and verbalizing subscales, while the affective dimension consists of emotionalizing and fantasizing (Vorst and Bermond, 2001). Based on these dimensions, different alexithymia types have been proposed (Bermond et al., 2007). Type-I alexithymia is characterized by high levels of both cognitive and affective alexithymia. Individuals with type-I alexithymia therefore experience lower levels of emotional arousal and fantasizing, together with impaired emotional cognition (e.g. difficulties in identifying, analyzing and verbalizing feelings). In contrast, type-II alexithymia is characterized by high levels of cognitive alexithymia together with low or normal levels of affective alexithymia. This indicates that individuals with type-II alexithymia have normal or heightened levels of

emotional arousal, while the cognitions accompanying these emotions (such as verbalizing and analyzing emotions) are impaired (for a schematic representation of the alexithymia dimensions and types, see Figure 1.1 in **chapter 1**). These alexithymia types and dimensions have been psychometrically identified (Bailey and Henry, 2007; Bermond et al., 2007; Vorst and Bermond, 2001). However, there has been some debate on whether they actually exist (Bagby et al., 2009). It has been previously suggested that the two alexithymia dimensions might be related to separable neural correlates, which could support the existence of these two dimensions (Bermond et al., 2006; Larsen et al., 2003; Wingbermühle et al., 2012). However, as outlined in **chapter 2**, most neuroimaging literature solely focused on the cognitive alexithymia dimension, because the most applied alexithymia questionnaire (TAS-20) does not assess the affective dimension (Bagby et al., 1994).

The structural and functional imaging results described in this thesis indicate that the two alexithymia dimensions are related to dissociable neural correlates. The cognitive dimension was related to lower activation in appraisal detection regions, such as the amygdala and visual cortex (**chapter 2** and **4**). This result is in agreement with the study of Pouga et al. (2010), the only other fMRI study examining the neural correlates of the two alexithymia dimensions. They reported an association between the cognitive dimension (e.g. identifying subscale) and lower amygdalar activation, which also indicates that the cognitive dimension might be specifically related to the aberrant activation during early appraisal detection in alexithymia. In line with this hypothesis, an EEG study of Goerlich et al. (2012) showed that aberrant early ERP components, related to appraisal detection, were associated with the cognitive dimension, but not the affective dimension.

In relation to the affective dimension, no aberrant activation patterns were found in this thesis (**chapter 4**). This finding is in contrast with the study of Pouga et al. (2010), who reported higher ACC and lower premotor cortex activation in association with the affective dimension. Surprisingly, the results of our meta-analysis (**chapter 2**) showed this pattern of higher ACC and lower premotor activation in relation to the cognitive dimension. One explanation for this could be that these activation patterns might be specifically related to type-I alexithymia (i.e. high levels on both the cognitive and affective dimension).

Besides the functional activation differences, the results of **chapter 3** revealed that the two alexithymia dimensions were also related to separable structural correlates. The affective alexithymia dimension was related to lower gray matter volume in the MOFC, which is suggested to be an emotion induction region (Rothkirch et al., 2012), and lower white matter volume in the superior longitudinal fasciculus, which is involved in fantasizing and imagination (Andrews Hanna et al., 2010; Makris et al., 2005). These structural abnormalities could be underlying the lower emotional arousal and lower levels of fantasizing in individuals with high affective alexithymia levels. Higher levels on the cognitive dimension, on the other hand, were related to lower dorsal ACC volume. Together with the recent finding of lower cingulate volume in association with lower levels of affective alexithymia (Goerlich-Dobre et al., 2014), this may point to a specific role of lower cingulate volume in type-II alexithymia (i.e. high levels of cognitive alexithymia and low or normal levels of affective alexithymia). This hypothesis of lower ACC volume in type-II alexithymia seems contradictory to the hypothesis of higher ACC activation in type-I alexithymia. However, lack of growth, atrophy or death of dendrites and neurons (possible reasons for lower ACC volume) do not necessarily imply lower activation, as the structural alterations may be associated with biochemical changes that enhance excitability, e.g. through disinhibition or compensatory activation of neighboring tissue. Future research should examine the relation between ACC volume and function in subjects specifically selected on type-I and type-II alexithymia.

Unraveling the neural correlates of the two alexithymia dimensions and alexithymia types is of great relevance as it has been shown that different patterns of scores on the two alexithymia dimensions might be related to different forms of psychopathology (Moormann et al., 2008a). For example, it was recently shown that somatoform disorders seem more related to type-I alexithymia, while borderline personality disorder was related to type-II alexithymia (Moormann et al., 2008a). Furthermore, type-II alexithymia has previously been related to schizophrenia (van 't Wout et al., 2007; van der Meer et al., 2009). In **chapter 5**, we confirmed that patients with schizophrenia indeed show a type-II alexithymia pattern. Furthermore, we found that the degree of vulnerability for psychosis was related to higher levels of cognitive alexithymia and lower or normal levels of affective alexithymia. Especially this type-II alexithymia pattern was related to higher levels of negative symptoms in the controls and siblings, which strengthens the idea that type-II alexithymia might be a vulnerability factor for schizophrenia.

Combined, these results indicate that alexithymia might not be a one-dimensional construct but rather seems to consist of at least two separable dimensions. Furthermore, these dimensions seem to be differently related to brain activation, brain structure and the vulnerability for psychopathology, such as schizophrenia.

|EMOTION REGULATION IN SUBJECTS AT HIGH RISK FOR PSYCHOSIS

Whereas emotion regulation difficulties have been well established in schizophrenia (see **chapter 1**), little to no research had yet been performed on emotion regulation in subjects at increased risk for psychosis. Therefore, in this thesis, we examined whether individuals at (putative) high-risk for developing psychosis differed from controls in the use and underlying neural correlates of two emotion regulation strategies, expressive suppression and cognitive reappraisal.

Expressive suppression

Expressive suppression (i.e. the inhibition of emotion-expressive behavior) is an emotion regulation strategy that has been associated with negative outcomes, such as lower levels of positive affect and life-satisfaction (Gross, 2002), and is therefore considered a less efficient emotion regulation strategy compared to reappraisal. Patients with schizophrenia appear to use expressive suppression more than controls (Kimhy et al., 2012; van der Meer et al., 2014). In this thesis, we examined whether subjects at increased risk for psychosis also report more use of expressive suppression.

The results revealed that only high levels of alexithymia were associated with more use of suppression (**chapter 4**), which is in line with previous reports (Kessler et al., 2010; Swart et al., 2009; Wingenfeld et al., 2011). Furthermore, the results of **chapter 2** showed that alexithymia is related to lower activation in MNS regions. These regions are involved in emotion expressive behavior (Carr et al., 2003) and recent research has shown that during suppression, activation in these regions is lower compared to performing mimicry (Vrticka et al., 2013). This may indicate that the reported higher frequency of suppression in alexithymia is related to lower MNS activation during emotion processing. Future research should correlate MNS activation to suppression in individuals with alexithymia. Furthermore, examining facial expression through electromyographic recordings in alexithymia and relating this to brain activation could give further insight in these relations.

Although siblings and UHR individuals did show higher levels of alexithymia (**chapter 5**), they did not report more use of suppression (**chapter 7** and **8**). This discrepancy is difficult to explain, however this finding corroborates the report of non-significant differences between siblings and controls on suppression in a smaller sample (van der Meer et al., 2014). It might be that instead of a general extensive use of suppression in these at risk groups, only siblings and UHR individuals with high levels of alexithymia apply more expressive suppression. However, more research is needed before conclusions can be drawn. Besides the absence of behavioral differences, no brain activation differences during expressive suppression were found between siblings and controls (**chapter 7**). Furthermore, alexithymia was not related to differential neural correlates during suppression (**chapter 4**). This finding is in agreement with the fact that no behavioral indications of difficulties in the use of suppression were found, i.e. all groups reported to use this strategy to the same extent or even more compared to controls and were capable of down-regulating negative affect through suppression.

Taken together, these results suggest that the extensive use of suppression might be an illness-related feature because it is found in patients with schizophrenia rather than in siblings and UHR individuals. However, as alexithymia was related to the use of suppression further research should examine the combined effect of higher levels of alexithymia and suppression on the risk for psychosis. Furthermore, we should note that self-report and peer-report measures of expressive suppression are only moderately correlated (John & Gross, 2003). Thus, it could be possible that subjects at high-risk were less capable of evaluating their own expressiveness. Therefore, future research should include peer assessments to evaluate the role of expressive suppression in individuals at risk for psychosis.

Cognitive reappraisal

In contrast to expressive suppression, the use of cognitive reappraisal has been related to positive outcomes such as higher levels of positive affect and well-being (Gross, 2002). Previous research has shown that patients with schizophrenia report less use of reappraisal compared to controls (Kimhy et al., 2012; Livingstone et al., 2009; van der Meer et al., 2009) and show lower prefrontal activation during reappraisal (Morris et al., 2012; van der Meer et al., 2014). We examined whether groups at high risk for psychosis also report less use of reappraisal and whether the neural correlates underlying reappraisal show signs of impairment in these groups.

The results showed that both siblings of patients with schizophrenia and subjects with high levels of alexithymia reported equal use of cognitive reappraisal as controls. The equal use of reappraisal in siblings further extents the non-significant findings on reappraisal in sibling reported by van der Meer et al. (2014), while the findings in alexithymia were in contrast with our expectations based on previous reports (Stasiewicz et al., 2012; Swart et al., 2009). However, research on the association between alexithymia and reappraisal are inconsistent as others also failed to show significant associations between alexithymia and reappraisal (Geenen et al., 2012; Weiss et al., 2012). Furthermore, the results did not show any aberrant brain activation patterns during cognitive emotion regulation in these groups (**chapter 4** and **7**). Moreover, no structural differences in prefrontal regulation regions were found (**chapter 3** and **6**). In contrast, individuals at UHR for psychosis did report less use of cognitive reappraisal compared to controls and showed lower activation of the VLPFC during reappraisal (**chapter 8**). The VLPFC is an important region during reappraisal as this region is responsible for the cognitive regulation of emotions (Ochsner et al., 2002) and related to reappraisal success (Wager et al., 2008). Therefore, lower activation during reappraisal in

this area might indicate emotion regulation difficulties in the UHR group. In a study of Modinos et al. (2010), the neural correlates of another high risk group for psychosis were studied during reappraisal. This risk group consisted of healthy students with elevated scores on schizotypy. The results revealed higher activation in the high schizotypy group in several regulation areas, among them the VLPFC. The authors suggested that this higher level of activation might have served as a compensatory mechanism (Modinos et al., 2010b).

Combined, these results show that in siblings and healthy individuals with high levels of alexithymia, the neural capacity to regulate emotions may be intact. Subjects with higher levels of schizotypy, but good overall functioning, are also still capable of cognitively regulating emotions, however they seem to need compensatory brain activation (Modinos et al., 2010b). When subclinical psychotic symptoms are combined with lower social functioning (UHR group), this compensatory mechanism appears to no longer work, resulting in lower prefrontal activation which may hamper the use of cognitive reappraisal.

Emotion dysregulation thus might be specific for subjects at UHR for psychosis and patients with schizophrenia. This substantiates the idea that emotion dysregulation may already occur in the prodromal phase of psychosis (Fowler et al., 2012). Emotion dysregulation is associated with lower social functioning, higher levels of negative affect and higher levels of anxiety (Gross, 2002). All of which are linked to the vulnerability for psychosis (Cornblatt et al., 2012; Fusar-Poli et al., 2014a). Furthermore, one could speculate that emotion dysregulation might play a role in delusion formation as previous research has shown that individuals with hallucinations in combination with high levels of negative affect have a greater chance of developing delusions (Hanssen et al., 2005; Krabbendam et al., 2005). This leads to the hypothesis that intact use of cognitive reappraisal might serve as a protective factor against psychosis.

|CONSIDERATIONS AND FUTURE IMPLICATIONS

Sample characteristics

In this thesis, three samples with a putatively increased risk for psychosis were examined. However, these groups are not solely at high risk for psychosis. For example, higher levels of alexithymia have also been found in autism (Berthoz et al., 2013), depression (Berthoz et al., 1999) and anxiety (Honkalampi et al., 2000). Furthermore, non-psychotic psychiatric disorders are more often found in siblings of patients with schizophrenia and UHR individuals compared to controls (Addington et al., 2012a; Maier et al., 2002). Therefore, the reported emotion processing and regulation abnormalities in this thesis might not be specific for the risk of psychosis, but may play a more general role in the development of psychopathology. Previous research has reported lower use of reappraisal in other psychiatric samples (e.g. pathological gamblers and depression) (Joormann and Gotlib, 2010; Williams et al., 2012). Furthermore, lower prefrontal activation during reappraisal has been reported in subjects at high risk for depression (Felder et al., 2012). This indicates that indeed emotion dysregulation might be a more general vulnerability factor for psychopathology. Future research should examine the association between specific symptoms and emotion dysregulation (and the neural correlates thereof) to gain insight into whether emotion dysregulation is related to specific or more general symptoms of psychopathology. Furthermore, performing longitudinal studies to examine whether emotion dysregulation is related to the conversion to psychosis could provide valuable information which may improve the prediction of a transition to psychosis.

Furthermore, siblings of patients with schizophrenia form a very heterogeneous sample. For example, some siblings may carry more genetic risk variants or encounter more traumatic life events, which makes them more vulnerable for psychosis than others. As outlined in **chapter 6** and **7**, this heterogeneity might explain the divergent and negative findings in this group. Selecting siblings based on specific characteristics (as performed in **chapter 6**), may provide insight into the underlying causes of these divergent findings. Furthermore, previous research has shown that different groups of siblings with different cognitive profiles can be distinguished (Quee et al., 2014). Future research should examine if it is also possible to divide siblings into groups with different affective functioning profiles.

In this thesis, alexithymia was assessed with a self-report inventory as this is the most common way to examine alexithymia. Self-report measures are reliant on reflecting one's own emotions, which is limited in individuals with alexithymia. Therefore, future research should include both self-report and observer-rated measures to assess alexithymia, such as the structured interview based on the Beth Israel Hospital Psychosomatic Questionnaire for alexithymia (Sriram et al., 1988). Furthermore, as outlined in this discussion, our results indicate that the two alexithymia dimensions are related to separable neural correlates. These two alexithymia dimensions can be used to distinguish separate types of alexithymia. Type-I alexithymia is related to high levels of both cognitive and affective alexithymia, which indicates that individuals experience low levels of emotional arousal and difficutlies with accompanying emotional cognitions. Type-II alexithymia, on the other hand, is related to high levels of cognitive alexithymia but normal or low levels of affective alexithymia, which indicates normal to high levels of emotional arousal but impaired emotional cognitions. These alexithymia types seem to be related to different forms of psychopathology (e.g. Moormann et al., 2008a; **chapter 5**). Therefore, studying the neural correlates of these different alexithymia types may provide information on the neural correlates underlying the risk for different forms of psychopathology. In this thesis, we took a first step by examining the neural correlates of the two alexithymia dimensions. However, future research should focus on selecting individuals with specifically type-I or type-II alexithymia to examine if the different combinations of alexithymia scores are indeed related to different neural correlates.

Task-related considerations

In **chapter 4**, **7** and **8**, an emotion regulation task was applied. Although this task consistently activated regions in accordance with previous literature, some considerations regarding this task should be mentioned.

All the emotion regulation tasks presented in this thesis applied a late cueing method in which the cue to reappraise was given after stimulus presentation. This method was chosen to allow subjects to have a naturalistic response to the emotional stimulus before regulation took place. However, this late cueing method may have caused the amygdala to habituate early (Ochsner et al., 2012), which could explain why we did not find any deactivation of the amygdala during reappraisal in our tasks. Future research should examine emotion regulation applying an early cueing paradigm to examine reappraisal in subjects at UHR for psychosis. This type of research could give insight into whether or not the lower prefrontal activation found during reappraisal in this group (**chapter 8**) is also related to less deactivation of the amygdala.

Second, research has shown that during reappraisal the activation in the prefrontal cortex is inversely correlated to amygdala activation (Banks et al., 2007). Unfortunately, we were unable to reliably study this fronto-limbic connection because the regulation blocks of

the emotion regulation task were too short (4s). Future research should therefore apply longer regulation times, or a block design, in order to examine functional connectivity patterns during reappraisal in individuals with an increased vulnerability for psychosis.

As mentioned in **chapter 1**, neuroimaging literature on expressive suppression is much more scarce compared to the literature on reappraisal. This is possibly due to the fact that correct application of the suppression strategy is difficult to control. Studying expressive suppression in individuals at high risk for psychosis, as well as in patients, is of great interest as these groups display fewer emotional expressions (Kring and Elis, 2013). Integrating electromyographic measurements or recording facial expression during scanning could provide more information on expressive suppression and the underlying neural correlates in both patients and subjects at high risk for psychosis.

The studies presented in this thesis only examined emotion regulation in lab-based settings. However, it would be interesting to examine whether our findings also translate to emotion regulation in daily life. Therefore, we suggest to apply momentary assessment studies on emotion regulation (as applied in Farmer and Kashdan, 2012) in subjects at increased risk for developing psychosis. It would be interesting to examine whether the lower VLPFC activation in the UHR group is related to emotion dysregulation in daily life. Furthermore, in this thesis we were unable to detect emotion dysregulation in individuals with high levels of alexithymia and siblings of patients with schizophrenia in a structured lab-setting. However, it is possible that in the more complex daily life without explicit instructions, these individuals do report some difficulties in emotion regulation.

In the meta-analysis of **chapter 2**, we showed that alexithymia was associated with lower activation in the insula and precuneus during the processing of positive stimuli. These results were suggested to be related to the lower positive affect reported by individuals with alexithymia. However, these results were based on a small number of studies, so further research on the neural correlates of positive emotion processing in alexithymia is needed. In general, neuroimaging research in the field of psychotic disorders has mainly focused on the processing and regulation of negative emotional stimuli, including the studies presented in this thesis. Although negative emotion processing is of great relevance in studying psychosis, possible impairments in positive emotion processing should not be overlooked. For example, anhedonia, the inability to experience pleasure from enjoyable events, is a symptom of schizophrenia. Furthermore, anhedonia has been reported in siblings of schizophrenia patients (Velthorst et al., 2012), UHR individuals (Valmaggia et al., 2013) and alexithymia (Tchanturia et al., 2012). It would be interesting to examine the relation between anhedonia and the ability to up-regulate positive emotions. Up-regulating positive affect is a cognitive emotion regulation strategy which increases the positive affect in response to positive stimuli (Giuliani et al., 2008). As this regulation strategy relies mainly on the same neural correlates as the reappraisal of negative events (Kim and Hamann, 2007), deficits in emotion regulation might also be underlying anhedonia in both patients with schizophrenia and individuals at increased risk for psychosis.

|CLINICAL IMPLICATIONS

The findings in this thesis show that the degree of risk for psychosis is associated with higher levels of alexithymia (**chapter 5**). Clinicians have occasionally reported individuals with alexithymia as treatment-resistant or avoidant because they do not seem to respond to psychodynamic psychotherapy treatments (Lumley et al., 2007; Taylor et al., 1997; Vanheule et al., 2011). Such treatment resistance is possibly not caused by unwillingness of the

alexithymic patient, but merely caused by an incapability of introspecting and verbalizing their feelings (Lumley et al., 2007; Taylor et al., 1997; Vanheule et al., 2011). Therefore, interventions designed for schizophrenia patients or subjects at increased risk for psychosis, should keep in mind that therapeutic interventions that rely on these processes might not work in these groups because of the higher levels of alexithymia. Rather, patients with high levels of alexithymia seem to better respond to more cognitive-behavioral treatments (Lumley et al., 2007; Vanheule et al., 2011). We therefore recommend cognitive-behavioral treatments in patients with schizophrenia and individuals at UHR for psychosis with high levels of alexithymia.

Our results indicate that UHR individuals may be impaired in the application of cognitive reappraisal (**chapter 8**). Although replication and further research is needed, this gives rise to the idea of implementing emotion regulation training in the UHR group. Previous research has shown that subjects at UHR for psychosis benefit from cognitive-behavioral therapy (CBT), as this results in lower transition rates (van der Gaag et al., 2012; van der Gaag et al., 2013) and lower levels of psychotic symptoms (Morrison et al., 2012). However, the levels of depression and anxiety do not change through CBT compared to treatment as usual (Morrison et al., 2012; van der Gaag et al., 2012). Combining CBT with a specific emotion regulation training has been shown to be effective in reducing negative affect and depression in a mixed psychiatric sample (Berking et al., 2008). Higher levels of negative affect are reported to precede psychosis (Fowler et al., 2012) and the UHR group shows aberrant emotion regulation patterns which could underlie these higher levels of negative affect (**chapter 8**). Therefore, including emotion regulation training in CBT might be a valuable addition to the interventions for individuals at UHR for psychosis.

|CONCLUDING REMARKS

Schizophrenia has long been viewed as a cognitive disorder. However, over the last two decades research has also focused on the emotion processing difficulties in schizophrenia (Aleman and Kahn, 2005). In this thesis, we showed that these emotion processing difficulties already occur in individuals at increased risk for developing psychosis. Schizophrenia and the vulnerability to this disorder are associated with higher levels of cognitive alexithymia which may impair early appraisal of emotional significance. Furthermore, in the UHR group emotion dysregulation may occur. Emotion dysregulation might contribute to poorer outcomes in this group as it is associated with psychopathology and poorer social dysfunction. Moreover, emotion dysregulation may lead to higher levels of negative affect which could increase the chance of a transition to psychosis. These results show that difficulties with emotion processing and emotion dysregulation are not solely related to schizophrenia or psychosis but may be part and parcel of the vulnerability for psychosis. This further substantiates the need of emotion-related research in psychotic disorders as this might result in better prediction of the transition to psychosis and could provide more targeted interventions.

PART IV

REFERENCES
ABBREVIATIONS
SAMENVATTING
DANKWOORD
LIST OF PUBLICATIONS
CURRICULUM VITAE
TOELICHTING OP VOORKANT

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ABBREVIATIONS

AAL	Automated anatomical labeling
ANOVA	Analysis of variance
ARMS	At-risk mental state
ACC	Anterior cingulate cortex
BLIPS	Brief limited intermittent psychotic syndrome
BVAQ	Bermond-Vorst alexithymia questionnaire
CAPE	Community assessment of psychic experiences
CAARMS	Comprehensive assessment of at risk mental states
CASH	Comprehensive assessment of symptoms and history
CHR	Clinical high risk
COMT	Catechol-O-methyltransferase
DARTEL	Diffeomorphic anatomical registration through exponentiated lie algebra
DLPFC	Dorsolateral prefrontal cortex
DMPFC	Dorsomedial prefrontal cortex
DSM	Diagnostic and statistical manual of mental disorders
EDIE-NL	Early detection and intervention evaluation
EEG	Electroencephalography
EHI	Edinburgh handedness inventory
ERQ	Emotion regulation questionnaire
FDR	False discovery rate
fMRI	Functional magnetic resonance imaging
FWE	Family-wise error
FWHM	Full width half maximum
GLM	General linear model
GM	Gray matter
GMV	Gray matter volume
GROUP	Genetic risk and outcome of psychosis
IAPS	International affective picture system
LEAS	Level of emotional awareness scale
METIGG	Mental healthcare research ethics committee
MNI	Montreal neurological institute
MNS	Mirror neuron system
MOFC	Medial orbitofrontal cortex
OCD	Obsessive compulsive disorder
OFC	Orbitofrontal cortex
PANAS	Positive and negative affect scale
PCC	Posterior cingulate cortex

PCM	Parametric coordinate-based meta-analysis
PQ-16	Prodromal questionnaire
SCAN	Schedules for the clinical assessment of psychiatry
SLF	Superior longitudinal fasciculus
SOFAS	Social and occupational functioning assessment scale
ROI	Region of interest
SVC	Small volume correction
TAS-20	Toronto alexithymia scale
UHR	Ultra-high risk
VBM	Voxel-based morphometry
VLPFC	Ventrolateral prefrontal cortex
WM	White matter
WMV	White matter volume

NEDERLANDSE SAMENVATTING

In het dagelijks leven krijgen we regelmatig te maken met situaties die negatieve gevoelens op kunnen roepen. Een lekke band kan bijvoorbeeld zorgen voor frustratie en drukte op het werk kan leiden tot stress. Hoe we op deze situaties reageren is afhankelijk van hoe we deze in eerste instantie waarnemen (emotieverwerking) en hoe goed we in staat zijn de gevoelens die we ervaren te controleren en reguleren (emotieregulatie). Emotieverwerking kan worden beschreven als het signaleren en verwerken van een emotionele stimulus en het ervaren van de bijbehorende positieve of negatieve emotie. Emotieregulatie, daarentegen, is het aanpassen van de emoties die je ervaart en het aanpassen van de manier waarop je deze laat zien. Er zijn verschillende vormen waarop emoties kunnen worden gereguleerd, maar in dit proefschrift staat herinterpreteren ("reappraisal") centraal. Herinterpreteren is het geven van een andere interpretatie aan een stimulus zodat deze minder negatief wordt. Stel, je ziet een huilende vrouw staan voor een kerk, dan zou je in eerste instantie kunnen denken dat er iets ergs gebeurd is. Echter na herinterpretatie kan je denken dat haar dochter net getrouwd is en dat het tranen zijn van geluk. Het gebruik van herinterpreteren heeft veel positieve effecten zoals het ervaren van meer positieve gevoelens en een hoger welzijn.

Als we geconfronteerd worden met een situatie die negatieve gevoelens opwekt, dan worden verschillende emotieverwerkingsgebieden in ons brein actief. Een van deze gebieden is de amygdala. De amygdala is ons emotionele waarschuwingssysteem en wordt actief zodra we met negatieve stimuli worden geconfronteerd. Dit gebied zorgt er vervolgens voor dat allerlei andere hersengebieden worden geactiveerd, zodat onze aandacht naar de emotionele stimulus gaat en we negatieve emoties ervaren. Als er emotieregulatie (e.g. herinterpretatie) plaatsvindt dan worden de prefrontale gebieden (voorste hersendelen) in ons brein actief. Deze hersengebieden zorgen voor het interpreteren en relativeren van de emotionele situaties. De prefrontale cortex zal er vervolgens voor zorgen dat de activatie in onder andere de amygdala weer afneemt zodat ook het negatieve gevoel weer vermindert.

Onderzoek heeft laten zien dat mensen met schizofrenie (voor een extra toelichting op schizofrenie, zie box 10.1) problemen ervaren met emotieverwerking en -regulatie. Zo is bijvoorbeeld de amygdala bij mensen met schizofrenie al actief in neutrale situaties. Dit kan er mogelijk voor zorgen dat mensen met schizofrenie neutrale situaties sneller als negatief interpreteren. Daarnaast maken mensen met schizofrenie minder gebruik van herinterpreteren en blijken ze minder goed in staat te zijn hun negatieve gevoelens te verminderen door middel van herinterpretatie. Ook blijkt de prefrontale cortex minder actief te zijn tijdens herinterpreteren in deze groep. Verminderde activatie in dit gebied is mogelijk onderliggend aan de emotieregulatieproblemen die mensen met schizofrenie ervaren.

Schizofrenie kan dus gerelateerd worden aan problemen in de emotieverwerking en emotieregulatie. Het is echter nog onduidelijk of deze problemen ontstaan als gevolg van de psychose, of dat deze problemen mogelijk ook al een rol spelen bij mensen met een verhoogd risico op een psychose. Het is belangrijk om hier onderzoek naar te doen, omdat dit inzicht kan geven in factoren die mogelijk het risico op het krijgen van een psychose kunnen vergroten. Aangezien schizofrenie een chronische stoornis is die lastig is te behandelen, is het belangrijk om deze risicofactoren te identificeren zodat deze verminderd kunnen worden ter preventie van psychoses.

Het doel van dit proefschrift was om te onderzoeken of de moeilijkheden met emotieverwerking en -regulatie al aanwezig zijn in mensen met een verhoogd risico op het ontwikkelen van een psychose. De nadruk lag hierbij op de neurale processen die zich afspelen in het brein tijdens emotieverwerking en -regulatie. Om dit te onderzoeken is er gekeken naar drie groepen met een verhoogd risico op het ontwikkelen van een psychose, namelijk mensen met een hoge score op alexithymie, broers en zussen van patiënten met

Box 10.1 Schizofrenie

Schizofrenie is een psychische aandoening welke wordt gekenmerkt door een grote variatie aan symptomen. Een belangrijk kenmerk van schizofrenie is het optreden van psychoses. Een psychose is een toestand waarin iemand het contact met de werkelijkheid gedeeltelijk of geheel verliest. Voorbeelden van symptomen die mensen tijdens een psychose kunnen hebben zijn hallucinaties, wanen (denkbeelden die niet overeenkomen met de werkelijkheid) en verward denken. Daarnaast tonen mensen met schizofrenie vaak minder initiatief, hebben ze minder interesse in sociale contacten en is de concentratie verminderd.
Schizofrenie is een chronische stoornis welke vaak moeilijk te behandelen is. De symptomen verergeren naarmate schizofrenie langer onbehandeld blijft. Het is daarom belangrijk om onderzoek te doen naar factoren die mogelijk een rol spelen bij het ontstaan van psychoses en schizofrenie. Dit onderzoek kan bijdragen aan de preventie en vroegdetectie van deze stoornissen.

schizofrenie en mensen met een ultrahoog risico op het ontwikkelen van een psychose. Deze drie risicogroepen worden verderop in deze samenvatting toegelicht.

Alexithymie

Alexithymie is een persoonlijkheidsdimensie welke letterlijk “een gebrek aan woorden voor emotie” betekent. Mensen met alexithymie hebben problemen met het onder woorden brengen, het identificeren en het analyseren van emoties. Daarnaast raken mensen met alexithymie minder snel geëmotioneerd en dagdromen/fantaseren ze minder. Mensen met schizofrenie scoren hoger op alexithymie (oftewel ze hebben meer alexithymie) in vergelijking met mensen zonder schizofrenie. Verder hebben mensen met een verhoogd risico op schizofrenie ook hogere alexithymie scores. Daarom kan alexithymie worden gezien als een mogelijke risicofactor voor het ontwikkelen van schizofrenie. Onderzoek naar alexithymie kan ons dus indicaties geven over welke processen mogelijk een rol spelen bij het ontwikkelen van een psychose.

In hoofdstuk 2 hebben we gekeken of het brein van mensen met alexithymie anders functioneert tijdens emotieverwerking dan dat van mensen zonder alexithymie. We hebben dit gedaan door te kijken wat hier al over bekend was in de literatuur. Door het uitvoeren van een meta-analyse, waarbij alle resultaten uit eerder onderzoek worden samengevoegd, werd gekeken welke resultaten het meest consistent in de literatuur worden gerapporteerd. Uit deze analyse kwam naar voren dat tijdens emotieverwerking, mensen met alexithymie een hogere activatie hebben in de anterieure cingulate cortex, een gebied belangrijk bij cognitieve emotieverwerking. Deze hogere activatie kan wijzen op een compensatiemechanisme, wat zou betekenen dat mensen met alexithymie extra activatie nodig hebben om emoties goed te kunnen verwerken. Daarnaast bleek dat alexithymie samenhangt met lagere activatie in een emotioneel aandachtssysteem (met o.a. de amygdala) en in gebieden die een belangrijke rol spelen bij empathie. Het emotioneel aandachtssysteem is verantwoordelijk voor het sturen van de aandacht naar emotionele stimuli. De lagere activatie bij mensen met alexithymie kan dus betekenen dat hun aandacht minder automatisch naar emotionele stimuli wordt getrokken. Dit zou kunnen verklaren waarom mensen met alexithymie moeite hebben met het identificeren van emoties. De gebieden die zijn betrokken bij empathie, waar ook verminderde activatie werd gevonden, zijn vooral belangrijk voor het signaleren van emoties bij anderen en voor het inleven in

anderen. Voor mensen met alexithymie is het inleven in anderen vaak lastig, wat dus mogelijk kan komen door deze verminderde activatie.

In eerder onderzoek is gesuggereerd dat alexithymie verdeeld kan worden in twee dimensies, een cognitieve en affectieve dimensie. Mensen met een hoge score op cognitieve alexithymie hebben problemen met het identificeren, verwoorden en analyseren van emoties. Terwijl mensen met een hoge score op de affectieve dimensie minder snel geëmotioneerd raken en minder fantaseren en dagdromen. Dit onderscheid is voorgesteld omdat mensen die moeite hebben om hun emoties te verwoorden, hier moeite mee kunnen hebben omdat ze weinig emoties ervaren (hoge score op cognitieve en affectieve dimensie). Maar, er zijn natuurlijk ook mensen die wel degelijk emoties ervaren, maar toch moeite hebben om deze te verwoorden (hoge score op alleen de cognitieve dimensie). Om te kijken of deze twee dimensies samenhangen met verschillende hersengebieden, hebben we in hoofdstuk 3 gekeken of de twee alexithymie dimensies gerelateerd zijn aan hersenvolume in verschillende hersengebieden. De resultaten toonden aan dat cognitieve alexithymie gecorreleerd was met een lager volume in de anterieure cingulate cortex, hetzelfde gebied dat compensatoire activatie liet zien in hoofdstuk 2. Lager volume in dit gebied, belangrijk bij cognitieve emotieverwerking, kan dus onderliggend zijn aan specifiek cognitieve alexithymie. Daarnaast bleek dat affectieve alexithymie gecorreleerd was met verminderd volume in de mediale orbitofrontale cortex en de superior longitudinale fasciculus (een baan die verschillende hersengebieden met elkaar verbindt). Dit verminderd volume kan mogelijk onderliggend zijn aan de verminderde ervaring van emoties en het verminderde fantaseren bij individuen met alexithymie aangezien deze gebieden daar een belangrijke rol in spelen.

Vanuit hoofdstuk 2 wisten we al dat alexithymie samenhangt met afwijkende activatiepatronen tijdens emotieverwerking. Het doel van hoofdstuk 4 was om te kijken of alexithymie ook gerelateerd is aan afwijkende hersenactivatie tijdens de latere fase van emotieregulatie. In overeenstemming met hoofdstuk 2 lieten de resultaten verminderde activatie zien in het emotioneel aandachtssysteem tijdens negatieve emotieverwerking. Er werden echter geen activatieverschillen gevonden tijdens emotieregulatie. Daarnaast bleken mensen met alexithymie even goed in staat om hun emoties te reguleren via herinterpretatie en gebruikten ze even veel herinterpretatie als mensen met lage scores op alexithymie. Deze resultaten laten zien dat er een verband is tussen alexithymie en vroege emotieverwerkingsproblemen, maar dat er geen verband is met emotieregulatieproblemen.

Tot slot is er in hoofdstuk 5 gekeken of alexithymie inderdaad samenhangt met het risico op het krijgen van een psychose, zoals in eerder onderzoek werd gesuggereerd. Om dit te onderzoeken werd alexithymie gemeten in broers en zussen van patiënten met schizofrenie, mensen met een ultrahoog risico op het ontwikkelen van een psychose en mensen met de diagnose schizofrenie. De resultaten lieten zien dat naarmate het risico op het krijgen van een psychose toenam, de score op de cognitieve dimensie van alexithymie hoger werd. Er kan dus worden geconcludeerd dat alexithymie inderdaad samenhangt met het risico op het ontwikkelen van een psychose.

Broers en zussen van mensen met schizofrenie

Broers en zussen van mensen met schizofrenie hebben een tien keer zo hoog genetisch risico op het krijgen van een psychose dan mensen zonder familieleden met schizofrenie. Eerder onderzoek heeft aangetoond dat mede door dit verhoogde genetisch risico, broers en zussen meer cognitieve functieproblemen hebben, zoals een minder goed functionerend werkgeheugen. Maar ook problemen met emotieverwerking lijken bij deze groep voor te

komen. Zo zijn broers en zussen van mensen met schizofrenie, als groep, minder goed in staat om emotionele gezichtsexpressies te identificeren. Dit komt mogelijk door het anders functioneren van het brein tijdens emotieverwerking, al is de exacte onderliggende neurale basis nog onduidelijk. In dit proefschrift hebben we daarom gekeken of broers en zussen minder hersenvolume hebben in gebieden die belangrijk zijn voor emotieverwerking (hoofdstuk 6). Tevens hebben we gekeken of broers en zussen ook afwijkende hersenactivatiepatronen laten zien tijdens emotieregulatie (hoofdstuk 7).

De resultaten van hoofdstuk 6 lieten zien dat broers en zussen van mensen met schizofrenie niet verschilden in hersenvolume van mensen zonder familieleden met schizofrenie. Dit kwam overeen met een aantal eerdere studies. Er zijn echter ook veel onderzoeken die wel verschillen in hersenvolume hebben gerapporteerd tussen deze groepen. Een mogelijke verklaring hiervoor zou kunnen zijn dat de groepen waarin niets gevonden werd al te oud waren. Schizofrenie ontwikkelt zich namelijk meestal voor het 30e levensjaar. Indien mensen deze leeftijd al voorbij zijn zal hun risico op het ontwikkelen van schizofrenie dus lager zijn. Dit zou er mogelijk voor kunnen zorgen dat er geen verschillen meer te zien zijn in hersenvolume. Verder zou het kunnen dat de onderzoeken die wel verschillen vonden toevallig mensen hadden geïncludeerd waarbij schizofrenie/psychoses vaker in de familie voorkwamen dan bij de andere studies en dat deze groepen daardoor een hoger risico hadden. In hoofdstuk 6 hebben we gekeken of het selecteren op leeftijd (onder de 30) of op familiaire belasting (meer dan 1 familielid met schizofrenie of een psychotische historie) uit zou maken voor de resultaten. Ook is er nog gekeken of de hoeveelheid lichte psychotische symptomen die de broers en zussen rapporteerden van invloed was. De resultaten lieten zien dat ook na het specifiek selecteren op deze factoren geen verschillen in hersenvolume werden gevonden tussen broers en zussen en de controlegroep. Hieruit kunnen we concluderen dat hersenvolume niet verschilt tussen mensen met en zonder genetisch risico op het ontwikkelen van schizofrenie.

In hoofdstuk 7 is er gekeken naar de hersenactivatie tijdens emotieregulatie bij zowel broers als zussen van mensen met schizofrenie. Uit de resultaten bleek dat de broers en zussen evenveel gebruik maakten van herinterpreteren als de controlegroep. Verder waren de broers en zussen even goed in staat om hun negatieve gevoel door middel van herinterpreteren te verminderen en werden er geen verschillen in hersenactivatie gevonden tijdens herinterpreteren. Hieruit kan worden geconcludeerd dat enkel het zijn van een broer of zus van iemand met schizofrenie niet lijkt te leiden tot problemen met emotieregulatie.

Ultrahoog risico op een psychose

De start van een psychose wordt vaak vooraf gegaan door een zogenaamde prodromale fase waarin individuen subklinische psychotische symptomen ervaren. Een voorbeeld van deze subklinische symptomen is het horen van je eigen stem hardop in je hoofd, terwijl je nog wel weet dat dit je eigen stem is. Er is hier dus nog geen sprake van een klinische psychose, maar mensen hebben vaak wel last van deze symptomen, bijvoorbeeld tijdens het concentreren. Deze subklinische psychotische symptomen gaan tijdens de prodromale fase vaak gepaard met een verminderd sociaal functioneren. Van individuen in deze prodromale fase wordt gedacht dat zij een ultrahoog risico (UHR) hebben op het ontwikkelen van een psychose. Na 2 jaar heeft ongeveer 29% van deze groep een psychose ontwikkeld. Jarenlang heeft de focus in het UHR-onderzoek gelegen op cognitieve processen. De laatste jaren is er echter ook steeds meer onderzoek gedaan naar emotionele processen. Zo blijken individuen met een UHR voor psychoses minder goed in staat om emoties te herkennen en laten ze een

verhoogde amygdala-activatie en een verlaagde prefrontale activatie zien in het brein tijdens emotieverwerking.

In hoofdstuk 8 hebben we gekeken of dit patroon ook zichtbaar was tijdens emotieregulatie. De resultaten lieten zien dat in de UHR-groep de activatie in de linker ventrolaterale prefrontale cortex (VLPFC, een gebied aan de linker voorzijde van het brein) verminderd actief was tijdens herinterpreteren. De VLPFC is een zeer belangrijk gebied voor emotieregulatie en zorgt ervoor dat emotionele situaties op een andere manier kunnen worden geïnterpreteerd. Verminderde activatie in dit gebied kan dus duiden op problemen met de emotieregulatie. Daarnaast rapporteerden mensen met een UHR op een psychose ook minder gebruik van herinterpretatie en een verhoogd negatief affect, wat de hypothese van problemen met emotieregulatie in deze groep ondersteunt.

Conclusie

De resultaten van dit proefschrift laten zien dat een verhoogd risico op het krijgen van een psychose inderdaad samenhangt met emotieverwerkingsproblemen in de vorm van een hogere score op alexithymie. Dit zou mogelijk ook kunnen leiden tot veranderde activatiepatronen in het brein zoals we zien bij alexithymie. Verder lieten de resultaten in dit proefschrift zien dat wanneer er sprake is van een licht verhoogd risico op het krijgen van een psychose (e.g. alexithymie of broers/zussen van mensen met schizofrenie) er geen sprake is van veranderingen in de neurale capaciteit om emoties te reguleren. Beide groepen lijken nog goed in staat om hun negatieve gevoel te verminderen en laten geen verminderde activatie zien tijdens emotieregulatie. Wel moet worden opgemerkt dat dit uiteraard alleen gemeten is in een experimentele setting, waarin emotieregulatie gestructureerd verliep en waarin mensen specifiek werden geïnstrueerd om hun emoties te reguleren. Er kunnen dus geen uitspraken worden gedaan over emotieregulatie in het dagelijks leven. Wanneer het risico op een psychose groter werd, zoals bij de UHR-groep, zagen we wel problemen met emotieregulatie. Deze groep lijkt een verminderde neurale capaciteit te hebben om emoties goed te kunnen reguleren en gebruiken dit daardoor ook minder.

De resultaten van dit proefschrift laten zien dat een verhoogd risico op het ontwikkelen van een psychose samenhangt met een verminderde capaciteit om emoties te verwerken en te reguleren. Vervolgonderzoek zal moeten uitwijzen of dit ook de kans op het krijgen van een psychose kan voorspellen.

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|CURRICULUM VITAE

Jorien was born on the 6th of April in Enschede, The Netherlands. In 1999 she started secondary school at the Stedelijk Lyceum Kottenpark in Enschede from which she graduated in 2005. Between 2005 and 2008 she studied Human Movement Sciences at the University of Groningen from which she obtained her Bachelor's degree in 2008. Due to her interest in the human brain, Jorien decided to switch to the field of neuropsychology, for which she followed a transitional programme. In 2009, Jorien started her master in Neuropsychology. Her Master's research project was carried out at the Neuroimaging Center of the University Medical Center of Groningen (UMCG). During this internship, she examined the neural correlates of emotion regulation in patients with schizophrenia under the supervision of dr. L. van der Meer and prof. dr. A. Aleman. In 2010, she graduated Cum Laude and obtained her Master of Science degree. Financed on a grant of the graduate school for Behavioural and Cognitive Neuroscience, Jorien started her PhD project leading to this thesis at the Neuroimaging Center of the UMCG. Her PhD project was supervised by prof. dr. A. Aleman, prof. dr. D. Wiersma and dr. R. Bruggeman. During the last year of her PhD project, Jorien started working at the Hanze University of Applied Sciences as a teacher in the department of Applied Psychology. At present, she continues working here.

|TOELICHTING OP DE VOORKANT

Een verward brein: niemand wil emoties van angst, verdriet, boosheid en verwarring in het brein. Als er te veel prikkels, te veel druk en stress is, te veel van alles eigenlijk, dan gaat het wel eens mis.

Maar wat gebeurt er dan precies? Ik zie het zo:

Je hersens komen in een cirkel die steeds groter wordt, omdat je overal over piekert en maalt. Je draait rond en net als in een tornado, sta je niet meer met je benen op de grond.

Het is chaos in je brein en die chaos, daar moet je uit. De negatieve en positieve emoties zijn uit balans. Donkere negatieve dingen dwalen in je hoofd en de lichte positieve dingen, verdwijnen naar de achtergrond. De tornado draait gewoon rond en rond en de zenuwen in je brein kunnen hier niet goed tegen.

Er moet weer balans en stabiliteit komen in het brein. Dan gaat de tornado weg.

Tenslotte na een zware storm komt er ook weer een fijne tijd.

Ilse van der Velde
2014, Enschede